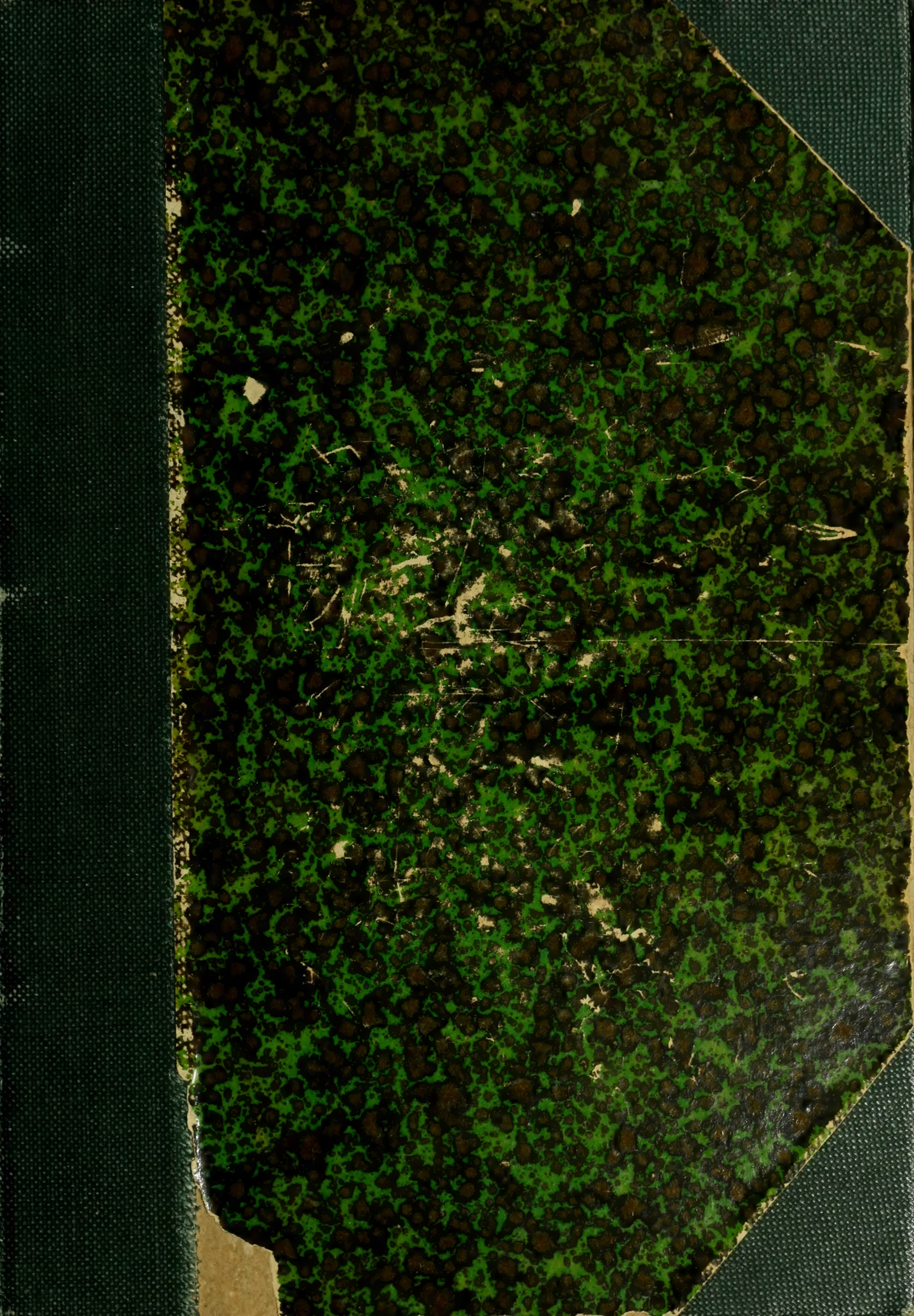

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SERIES B

CONTAINING PAPERS OF A BIOLOGICAL CHARACTER

VOL. LXXXII.

LONDON:
PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON AND SONS, ST. MARTIN'S LANE,
PRINTERS IN ORDINARY TO HIS LATE MAJESTY.

SEPTEMBER, 1910.

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HARRISON AND SONS, PRINTERS IN ORDINARY TO HIS LATE MAJESTY,
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CONTENTS.

SERIES B. VOL. LXXXII.

No. B 552.—December 20, 1909.

PAGE

Experimental Researches on Vegetable Assimilation and Respiration. V.—A Critical Examination of Sachs' Method for using Increase of Dry Weight as a Measure of Carbon Dioxide Assimilation in Leaves. By D. Thoday, M.A., Mackinnon Student of the Royal Society, late Frank Smart Student of the University of Cambridge. Communicated by Dr. F. F. Blackman, F.R.S. 1

Sleeping Sickness in Uganda.—Duration of the Infectivity of the *Glossina palpalis* after the Removal of the Lake-shore Population. By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908–09) 56

Glossina palpalis as a Carrier of *Trypanosoma vivax* in Uganda. By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908–09) 63

On the Presence of Hæm-agglutinins, Hæm-opsonins, and Hæmolysins in the Blood obtained from Infectious and Non-Infectious Diseases in Man. (Third Report.) By Leonard S. Dudgeon, F.R.C.P. Lond., and H. A. F. Wilson. Communicated by Dr. F. W. Mott, F.R.S. 67

No. B 553.—January 14, 1910.

The Germicidal Action of Metals and its Relation to the Production of Peroxide of Hydrogen. By Allan C. Rankin, M.D., Demonstrator in Bacteriology, McGill University, and Assistant in Bacteriology, Royal Victoria Hospital, Montreal. Communicated by J. G. Adami, F.R.S. 78

Gametogenesis of the Gall-Fly, *Neuroterus lenticularis* (*Spathegaster baccarum*).—Part I. By Leonard Doncaster, M.A., Lecturer on Zoology, Birmingham University. Communicated by Prof. W. Bateson, F.R.S. (Plates 1–3) 88

Preliminary Note upon the Cell Lamination of the Cerebral Cortex of Echidna, with an Enumeration of the Fibres in the Cranial Nerves. By Edgar Schuster, D.Sc., Fellow of New College (Pathological Laboratory, Claybury Asylum, Essex, and Department of Comparative Anatomy, University Museum, Oxford). Communicated by Dr. F. W. Mott, F.R.S. (Plates 4 and 5) 113

PAGE

Cortical Lamination and Localisation in the Brain of the Marmoset. By F. W. Mott, M.D., F.R.S.; E. Schuster, D.Sc.; and W. D. Halliburton, M.D., F.R.S. (Plates 6 and 7) 124

The Caudal Fin of Fishes (Preliminary Paper). By Richard H. Whitehouse, M.Sc. Birm. Communicated by Prof. F. W. Gamble, D.Sc., F.R.S. 134

Some Experiments with the Venom of *Causus rhombeatus*. By H. E. Arbuckle, M.B., West African Medical Service. Communicated by A. Alcock, C.I.E., F.R.S. 144

On the Comparative Action of Stovaine and Cocaine as measured by their Direct Effect upon the Contractility of Isolated Muscle. By V. H. Veley, F.R.S., and A. D. Waller, F.R.S. 147

No. B 554.—February 3, 1910.

Address of the President, Sir Archibald Geikie, K.C.B., at the Anniversary Meeting on November 30, 1909 151

The Velocity of Reaction in the "Absorption" of Specific Agglutinins by Bacteria, and in the "Adsorption" of Agglutinins, Trypsin, and Sulphuric Acid by Animal Charcoal. By Georges Dreyer, M.A., M.D., Professor of Pathology in the University of Oxford; and J. Sholto C. Douglas, M.A., B.M., Philip Walker Student in Pathology in the University of Oxford, formerly Radcliffe Travelling Fellow. Communicated by Prof. F. Gotch, F.R.S. 168

On the Absorption of Agglutinin by Bacteria and the Application of Physico-chemical Laws thereto. By Georges Dreyer, M.A., M.D., Professor of Pathology in the University of Oxford; and J. Sholto C. Douglas, M.A., B.M., Philip Walker Student in Pathology in the University of Oxford, formerly Radcliffe Travelling Fellow. Communicated by Prof. F. Gotch, F.R.S. 185

Observations on the Rate of Action of Drugs (Alcohol, Chloroform, Quinine, Aconitine) upon Muscle as a Function of Temperature. By V. H. Veley, F.R.S., and A. D. Waller, M.D., F.R.S. 205

An Examination of the Physical and Physiological Properties of Tetrachlorethane and Trichlorethylene. By V. H. Veley, F.R.S. 217

The Photochemical Formation of Formaldehyde in Green Plants. By S. B. Schryver, D.Sc., Ph.D., Lecturer on Physiological Chemistry in University College, London. Communicated by Prof. E. H. Starling, F.R.S. 226

No. B 555.—March 3, 1910.

Further Observations on the Pathology of Gastric Ulcer. (Progress Report.) By Charles Bolton, M.D., D.Sc., F.R.C.P., Director of the Research Laboratories, University College Hospital Medical School, Assistant Physician to University College Hospital. Communicated by Prof. Sidney Martin, F.R.S. (Plates 8 and 9) 233

On the Action of Antimony Compounds in Trypanosomiasis in Rats: being a Report to a Sub-Committee of the Tropical Diseases Committee of the Royal Society. By John D. Thomson, M.B., C.M., and Arthur R. Cushny, M.D., F.R.S. 249

PAGE

Amakebe : A Disease of Calves in Uganda. By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service ; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps ; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908-09.) (Plate 10) 256

The Transmission of *Trypanosoma lewisi* by the Rat-flea (*Ceratophyllus fasciatus*). (Preliminary Communication.) By E. A. Minchin, M.A., and J. D. Thomson, M.A., M.B., C.M. Communicated by Sir Ray Lankester, K.C.B., F.R.S.......... 273

On the Relative Sizes of the Organs of Rats and Mice bearing Malignant New Growths. By Dr. F. Medigreceanu (Bucharest). Communicated by Dr. J. Rose Bradford, Sec. R.S. 286

The Contrast in the Reactions to the Implantation of Cancer after the Inoculation of Living and Mechanically Disintegrated Cells. By M. Haaland, M.D., Imperial Cancer Research Fund. Communicated by Dr. J. Rose Bradford, Sec. R.S. 293

Further Evidence on the Homogeneity of the Resistance to the Implantation of Malignant New Growths. By E. F. Bashford, M.D., and B. R. G. Russell, M.D., Imperial Cancer Research Fund. Communicated by Dr. J. Rose Bradford, Sec. R.S. 298

No. B 556.—May 10, 1910.

Contributions to the Biochemistry of Growth.—The Total Nitrogen Metabolism of Rats bearing Malignant New Growths. By W. Cramer and Harold Pringle. Communicated by Prof. E. A. Schäfer, F.R.S. 307

Contributions to the Biochemistry of Growth.—Distribution of Nitrogenous Substances in Tumour and Somatic Tissues. By W. Cramer and Harold Pringle. Communicated by Prof. E. A. Schäfer, F.R.S. 315

The Alcoholic Ferment of Yeast-juice. Part V.—The Function of Phosphates in Alcoholic Fermentation. By Arthur Harden, F.R.S., and William John Young (Biochemical Laboratory of the Lister Institute of Preventive Medicine) 321

The Causes of Absorption of Oxygen by the Lungs. (Preliminary Communication.) By C. Gordon Douglas, B.M., Fellow of St. John's College, and J. S. Haldane, M.D., F.R.S., Fellow of New College, Oxford 331

The Action of Nicotine and other Pyridine Bases upon Muscle. By V. H. Veley, F.R.S., and A. D. Waller, F.R.S. 333

Preliminary Note on the Origin of the Hydrochloric Acid in the Gastric Tubules. By Mabel Purefoy FitzGerald (Oxford). Communicated by Prof. A. B. Macallum, F.R.S. 346

Studies on Enzyme Action. XIII.—Enzymes of the Emulsin Type. By Henry E. Armstrong, F.R.S., and Edward Horton, B.Sc. 349

The Development of Trypanosomes in Tsetse Flies. By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service ; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps ; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908–10) 368

PAGE

No. B 557.—July 21, 1910.

The Thyroid and Parathyroid Glands throughout Vertebrates. By F. D. Thompson. Communicated by Prof. E. A. Schäfer, F.R.S. (Abstract) 389

Tone Perception in *Gammarus pulex*. By F. J. Cole, B.Sc., Professor of Zoology, University College, Reading. Communicated by A. E. Shipley, M.A., F.R.S.... 391

A Physiological Effect of an Alternating Magnetic Field. By Silvanus P. Thompson, D.Sc., F.R.S. 396

On the Weight of Precipitate Obtainable in Precipitin Interactions. By Dr. H. G. Chapman. Communicated by Dr. C. J. Martin, F.R.S. 398

The Influence of Bacterial Endotoxins on Phagocytosis. (Preliminary Report.) By Leonard S. Dudgeon, P. N. Panton, and H. A. F. Wilson. Communicated by Dr. F. W. Mott, F.R.S........................ 406

A Case of Sleeping Sickness Studied by Precise Enumerative Methods: Regular Periodical Increase of the Parasites Disclosed. By Major Ronald Ross, F.R.S., and David Thomson, M.B., Ch.B., D.P.H. 411

On the Recognition of the Individual by Hæmolytic Methods. (Preliminary Communication.) By Charles Todd, M.D., Bacteriologist, Egyptian Government, and R. G. White, M.B., Director, Serum Institute, Cairo. Communicated by Dr. C. J. Martin, F.R.S. 416

Experimental Researches on Vegetable Assimilation and Respiration. VI.—Some Experiments on Assimilation in the Open Air. By D. Thoday, M.A., Junior University Demonstrator in Botany, Cambridge. Communicated by Dr. F. F. Blackman, F.R.S. 421

Receptors and Afferents of the Third, Fourth, and Sixth Cranial Nerves. By Frances M. Tozer, B.Sc., and C. S. Sherrington, D.Sc., F.R.S. 450

The Relation of Light Perception to Colour Perception. By F. W. Edridge-Green, M.D., F.R.C.S., Beit Medical Research Fellow. Communicated by Prof. E. H. Starling, F.R.S. 458

No. B 558.—August 10, 1910.

Trypanosome Diseases of Domestic Animals in Uganda. I.—*Trypanosoma pecorum*. By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908–9). (Plates 11 and 12)........................ 468

Experiments to ascertain if Cattle may act as a Reservoir of the Virus of Sleeping Sickness (*Trypanosoma gambiense*). By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908–10) 480

"Muhinyo," a Disease of Natives in Uganda. By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908–10) 485

PAGE

The Natural Food of *Glossina palpalis*. By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10) 490

Mechanical Transmission of Sleeping Sickness by the Tsetse Fly. By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10) 498

The Chromaphil Tissues and the Adrenal Medulla. By Swale Vincent, M.D., D.Sc., F.R.SS. E. and C., Professor of Physiology in the University of Manitoba. Communicated by Prof. E. A. Schäfer, F.R.S. (Plate 13) 502

On the Occurrence of a Mesocœlic Recess in the Human Brain, and its Relation to the Sub-Commissural Organ of Lower Vertebrates; with special reference to the Distribution of Reissner's Fibre in the Vertebrate Series and its possible Function. By Arthur Dendy, D.Sc., F.R.S., Professor of Zoology in King's College (University of London), and G. E. Nicholls, B.Sc., Assistant-Lecturer and Demonstrator in Zoology in King's College. (Plate 14) 515

Autotoxæmia and Infection. By E. C. Hort, F.R.C.P. Ed., Assistant Physician to the Italian Hospital. Communicated by William Osler, F.R.S. 529

The Blood Volume of Mammals as Determined by Experiments upon Rabbits, Guinea-pigs, and Mice, and its Relationship to the Body Weight and to the Surface Area expressed in a Formula. By Georges Dreyer, M.A., M.D., and William Ray, B.Sc., M.B. Communicated by Prof. F. Gotch, F.R.S. (Abstract) 545

No. B 559.—September 9, 1910.

Croonian Lecture.—Alterations in the Development and Forms of Plants as a Result of Environment. By Prof. G. Klebs, University of Heidelberg 547

The Origin and Destiny of Cholesterol in the Animal Organism. Part VII.—On the Quantity of Cholesterol and Cholesterol Esters in the Blood of Rabbits Fed on Diets containing Varying Amounts of Cholesterol. By Mary T. Fraser, B.Sc., and J. A. Gardner, M.A. Communicated by Dr. A. D. Waller, F.R.S. 559

On the Comparative Toxicity of Theobromine and Caffeine, as Measured by their Direct Effect upon the Contractility of Isolated Muscle. By V. H. Veley, F.R.S., and A. D. Waller, M.D., F.R.S. 568

A New Method for the Quantitative Estimation of Hydrocyanic Acid in Vegetable and Animal Tissues. By Dr. A. D. Waller, F.R.S. 574

The Origin of Osmotic Effects. III.—The Function of Hormones in Stimulating Enzymic Change in Relation to Narcosis and the Phenomena of Degenerative and Regenerative Change in Living Structures. By Henry E. Armstrong, F.R.S., and E. Frankland Armstrong, Ph.D., D.Sc. 588

The Inorganic Composition of the Blood in Vertebrates and Invertebrates, and its Origin. By Prof. A. B. Macallum, Ph.D., Sc.D., F.R.S., of the University of Toronto 602

PAGE

The Anatomy and Morphology of the Leaves and Inflorescences of *Welwitschia mirabilis*. By Miss M. G. Sykes. Communicated by Prof. A. C. Seward, F.R.S. (Abstract) 625

No. B 560.—September 23, 1910.

The Assimilation of Nitrogen by certain Nitrogen-fixing Bacteria in the Soil. By W. B. Bottomley, M.A., Professor of Botany in King's College, London. Communicated by Prof. J. Reynolds Green, F.R.S. 627

On the Structure, Development, and Morphological Interpretation of the Pineal Organs and Adjacent Parts of the Brain in the Tuatara (*Sphenodon punctatus*). By Arthur Dendy, D.Sc., F.R.S., Sec.L.S., Professor of Zoology in King's College (University of London). (Abstract) 629

The Opposite Electrification produced by Animal and Vegetable Life. By W. M. Thornton, D.Sc., D.Eng., Professor of Electrical Engineering in Armstrong College, Newcastle-upon-Tyne. Communicated by Dr. A. D. Waller, F.R.S. ... 638

The Fermentation of Galactose by Yeast and Yeast-juice. (Preliminary Communication.) By Arthur Harden, F.R.S., and Roland V. Norris 645

The Lignite of Bovey Tracey. By Clement Reid, F.R.S., and Eleanor M. Reid, B.Sc. (Abstract) 650

Obituary Notices of Fellows Deceased:—E. F. W. Pflüger; William Henry Dallinger; Thomas William Bridge .... i—x

Index xi

4888

PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 82. **No. B 552.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page

Experimental Researches on Vegetable Assimilation and Respiration. V.—A Critical Examination of Sachs' Method for using Increase of Dry Weight as a Measure of Carbon Dioxide Assimilation in Leaves. By D. THODAY, M.A., Mackinnon Student of the Royal Society, late Frank Smart Student of the University of Cambridge 1

Sleeping Sickness in Uganda.—Duration of the Infectivity of the Glossina palpalis after the Removal of the Lake-shore Population. By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908—09) 56

Glossina palpalis as a Carrier of Trypanosoma vivax in Uganda. By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908—09) 63

On the Presence of Hæm-agglutinins, Hæm-opsonins, and Hæmolysins in the Blood obtained from Infectious and Non-Infectious Diseases in Man. (Third Report.) By LEONARD S. DUDGEON, F.R.C.P. Lond., and H. A. F. WILSON 67

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Two Shillings and Sixpence.

No. B 552.

December 20, 1909.

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PROCEEDINGS OF

THE ROYAL SOCIETY.

Section B.—Biological Sciences.

Experimental Researches on Vegetable Assimilation and Respiration. V.—A Critical Examination of Sachs' Method for using Increase of Dry Weight as a Measure of Carbon Dioxide Assimilation in Leaves.*

By D. Thoday, M.A., Mackinnon Student of the Royal Society, late Frank Smart Student of the University of Cambridge.

(Communicated by Dr. F. F. Blackman, F.R.S. Received June 11,—Read June 24, 1909.)

CONTENTS.

		PAGE
Section	I.—Introduction	1
,,	II.—On the Nature of the Errors to which the Half-leaf Dry-Weight Method is Liable	3
,,	III.—On Changes of Composition during Insolation	4
,,	IV.—The Error from Lack of Symmetry	12
,,	V.—The Error from Change of Area during Experiment	19
,,	VI.—On the Measurement of Area	39
,,	VII.—On the Killing and Drying of Leaf Material	45
,,	VIII.—General Considerations on the Degree of Accuracy and the Utility of the Method	48
,,	IX.—Conclusions	53

Section I.—Introduction.

The trustworthiness of Sachs' well-known dry-weight method[†] for measuring the rate of accumulation and translocation of the products of

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[*] Earlier contributions to this series of investigations carried out under the general direction of Dr. F. F. Blackman at Cambridge are the following: I and II, Blackman, 'Phil. Trans.,' B, 1895; III, Matthaei, 'Phil. Trans.,' B, 1904; and IV, Blackman and Matthaei, 'Roy. Soc. Proc.,' B, 1905.

[†] Sachs, Julius von, "Ein Beitrag zur Kenntniss der Ernährungsthätigkeit der Blätter," 'Arbeit. d. Bot. Inst. in Würzburg,' III, 1883, p. 19.

photosynthesis in leaves was called in question in 1905 by Brown and Escombe. In their paper "On the Physiological Processes of Green Leaves,"* they published an account of four experiments in which they had determined for the same individual leaves both the increase of dry weight, by Sachs' method, and the amount of carbon dioxide actually absorbed, by their own method. They sum up the results by saying: "If we take the mean of all four experiments we find that the Sachs method gives an estimate of the assimilation rate between two and three times greater than that deduced from the intake of carbon dioxide."†

In attempting to explain this discrepancy they made some determinations of the degree of symmetry existing between opposite sides of various leaves, and of the amount by which half-leaves changed in area under experimental conditions. They concluded from these that the errors to which the method is liable are of the same order of magnitude as the quantities to be measured, and that therefore "the Sachs method cannot be trusted for anything like exact quantitative estimation of the photosynthetic work which is going on in an assimilating leaf. As ordinarily applied, its general tendency is to give far too high an estimate of the rate of assimilation."‡

Since Sachs' method is the only one available for measuring the total photosynthetic activity of leaves under *natural conditions*, it is of the utmost importance that it should not lightly be abandoned. Its loss would be felt in pure physiology, and perhaps still more in ecology. I therefore undertook, at Dr. F. F. Blackman's suggestion, a detailed investigation of the Sachs method, in order to discover whether it was completely untrustworthy, or whether its weaknesses could be overcome.

Since the present paper is concerned principally with the results of this examination of the method, much is included that is technical and of importance only to those who wish to use the method;§ but the nature of the changes occurring in the leaf lamina during insolation (Section III) and the phenomena of shrinkage and expansion in area which follow variations in the intensity of illumination (Section V) are subjects of more general interest. Some applications of the method will be described in a later paper.

The work was carried out in the Cambridge University Botany School during my tenure of the Cambridge University Frank Smart Studentship. My thanks are due to Dr. F. F. Blackman for his kind interest in the work throughout its course and for many helpful suggestions.

---

* 'Roy. Soc. Proc.,' B, vol. 76, 1905, p. 29.

† *Loc. cit.*, p. 58.

‡ *Loc. cit.*, p. 61.

§ The most technical portions are in smaller type.

Section II.—On the Nature of the Errors to which the Half-leaf Dry-Weight Method is Liable.

The Sachs method consists essentially in the comparison of the dry weight per unit area of one half of a leaf (the control half-leaf) at the beginning of an experiment, with that of the other (the experimental half-leaf) after a certain number of hours of assimilation. The difference is taken to be the weight of the products which have been accumulated by the leaf during that time per unit of area.

Underlying the method are two assumptions, (1) that the symmetry is perfect, *i.e.* that the two halves of every leaf used possess appreciably the same dry weight per unit area, and (2) that no change takes place in the experimental half leaf to alter the original dry weight per unit area except the accumulation of the products of photosynthesis which it is the object of the experiment to measure.

The few experiments of Brown and Escombe clearly prove these assumptions to be unjustifiable, as leaves are far from symmetrical, and leaf area may change under experimental conditions. Further, to help explain the recorded excessive gain of dry weight during assimilation, they suggest a possible increase in the retention of water by colloids of the insolated leaf.*

If increase of dry weight were partly due to such an indeterminable fixation of water, the increase could not be used as a measure of assimilation, and the whole procedure would fall to the ground. This fear will be shown in Section III to be without foundation.

Catalpa bignonioides, used by Brown and Escombe in their test of the dry-weight method, happens to have been an unfortunate choice, since its leaves are particularly unsymmetrical in respect of dry weight. Other leaves show a much closer agreement between the two halves. The degree of asymmetry of a number of species is dealt with in Section IV.

Change of area has proved to be of extreme importance, as it is to errors from this source that the observed tendency to high results is due. Section V is devoted to this question.

In later sections of the paper minor errors of technique are carefully considered and estimated. Errors in the measurement of area, errors in weighing due to incomplete drying of the hygroscopic leaf material, etc., become of real importance, because their effects are cumulative in the resulting error in the observed gain of dry weight.

The following is a formal analysis of the various sources of error. Of those classed as errors of interpretation, that due to the varying composition of the increase is briefly dealt with in Section III; the others are outside the scope of the present paper:—

---

* *Loc. cit.* p. 59.

Summary of Sources of Error affecting the Half-leaf Dry Weight Method.

i. Errors introduced in any single case in estimating the dry weight of the unit of area of leaf lamina:
  a. In measuring or determining the area, by the various methods Section VI.
  b. In determining dry weight:
    (1). In drying .... Section VII.
    (2) In weighing.

ii. Errors introduced in the comparison of two halves of a leaf:
  a. Through asymmetry due to:
    (1) Unsymmetrical venation; } .... Section IV.
    (2) Inequalities in thickness; }
    (3) Differences in composition........................ Section III.
  b. Through changes taking place in the course of experiment:
    (1) In area .... Section V.
    (2) In composition, including change in power of retaining water when dried .... Section III.

iii. Errors affecting the interpretation of the observed increase, due to:
  a. The heterogeneous composition of the increase—starch, sugars, proteids, oils, etc. .... Section III.
  b. The occurrence of respiration and translocation.

Section III.—On Changes of Composition during Insolation.

To ascertain whether any of the observed increase of dry weight during insolation is due to fixation of water, the *gain of carbon* per unit of area was determined in the same half-leaves in which the increase of dry weight had been carefully measured. For this purpose the "experimental" and "control" pieces of leaf were analysed by combustion, and the gain in carbon content per unit of area determined by the difference.

The analyses were carried out in the Caius College Chemical Laboratory, and my thanks are due to Dr. Ruhemann, of Caius College, for placing his apparatus at my disposal, and giving me every facility.

If photosynthesis resulted in the accumulation of some known single substance, the increase of dry weight could be accurately calculated from the gain of carbon. As, however, starch, various sugars, proteids, and even oils may be produced, any basis for calculation must be in some degree arbitrary, and perfect correspondence between the calculated increase and that actually observed is not to be expected.

There are, besides, other minor sources of discrepancy. The apparent gain of dry weight which would result from any shrinkage in area of the experimental half-leaf would have the composition of the general leaf substance, and this might be quite different from the composition of the true assimilatory increase. Experimental errors also are not negligible.

If, with all these disturbing factors, a close agreement holds between the observed increase of dry weight and the values calculated from the results of the carbon analyses, it is clear that fixation of water cannot play an appreciable part, still less produce an overwhelming positive error over and above the real assimilatory gain.

Errors from incomplete drying may be for the present regarded as negligible. They are considered in Section VII (p. 45), where the procedure finally adopted for drying the very hygroscopic material is described.

The following tables give the results of the combustion analyses and the dry-weight results with which they are to be compared :—

In Tables I to III the calculated value for the gain of dry weight has been obtained by finding the weight of starch which is equivalent to the observed gain of carbon, and adding to this the observed increase in the ash content.

As the ash consists largely of carbonate, a few specimens were analysed, using lead chromate to decompose the carbonates of the alkali metals not decomposed by heat. Tables IV and V give the results obtained. The calculated value for the increase of dry weight is there simply the starch equivalent of the observed gain of carbon.

The essentials of the conditions under which the material analysed was obtained are given above each table.

The examples of *Helianthus tuberosus* in Table I were carefully powdered before the final drying and combustion. The results in the other tables were obtained with material which had been dried in a form convenient for immediate analysis.*

The entire half-leaves were employed for the experiments in Table I and their area found by applying a planimeter to photographic prints ;† the results are therefore all calculated for an area of one square decimetre.

Where equal areas from each half-leaf were cut, by means of templets,† such as were used by Sachs, or by the rotating punch† described on p. 42, no such calculation was necessary, and for convenience in estimating the degree of accuracy the results have been left in terms of the actual area used. This area is given for each experiment.

Where the punch method was used the number of pieces punched from each half-leaf is given ; each piece measured 0·804 square centimetre. In one or two cases the numbers of discs from the two halves were not identical, and in these examples the figures for one half are calculated for the number of discs used in the other half.

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* See p. 45.

† The various methods of area determination are described in Section VII. For brevity they will be referred to as the planimeter, templet, and punch methods respectively.

Table I.—*Helianthus tuberosus*: area of entire half-leaves by planimeter method.

Assimilation experiment; August 23, 1906. Leaves attached to plant; darkened previous evening. Intermittent sun. Time, 7½ hours: 10 A.M. to 5.30 P.M. *a* = control half-leaf; *b* = experimental half-leaf.

Numbers are grammes per square decimetre.

Leaf.	Area in sq. cm.	Dry weight.	Gain of dry weight.	CO_2 found on combustion.	Gain of CO_2.	Starch corresponding to gain of CO_2.	Ash found on combustion.	Gain of ash.	Calculated gain of dry weight.
1	(*a*) 26·9 (*b*) 26·8	0·378 0·417	**0·039**	0·633 0·694	0·061	0·037	0·049 0·053	0·004	**0·041**
2	(*a*) 60·2 (*b*) 58·5	0·342 0·365	**0·023**	0·556 0·603	0·047	0·029	0·040 0·043	0·003	**0·032**
3	(*a*) 77·5 (*b*) 85·5	0·399 0·393	**−0·006**	0·618 0·611	−0·007	−0·005	0·064 0·063	−0·001	**−0·006**
4	(*a*) 80·2 (*b*) 77·8	0·446 0·470	**0·024**	0·671 0·704	0·033	0·019	0·084 0·086	0·002	**0·021**

Table II.—*Helianthus tuberosus*: area by templet method.

Assimilation experiment; August 28, 1907. Leaves detached, in greenhouse; temperature 25°—27° C. Sun, under canvas. Time 8.30 A.M. to 3.30 P.M.: 7 hours.

Numbers are grammes, and refer to the given areas.

Leaf.	Area in sq. cm.	Dry weight.	Gain of dry weight.	CO_2 found on combustion.	Gain of CO_2.	Starch corresponding to gain of CO_2.	Ash found on combustion.	Gain of ash.	Calculated gain of dry weight.
1	50	(*a*) 0·1465 (*b*) 0·1668	**0·0203**	0·2324 0·2665	0·0361	0·0209	0·0236 0·0267	0·0031	**0·0240**
2	50	(*a*) 0·1513 (*b*) 0·1622	**0·0109**	0·2518 0·2651	0·0133	0·0082	0·0195 0·0219	0·0024	**0·0106**
3	50	(*a*) 0·1449 (*b*) 0·1597	**0·0148**	0·2333 0·2556	0·0223	0·0137	0·0236 0·0267	0·0031	**0·0168**
4	40	(*a*) 0·1223 (*b*) 0·1297	**0·0074**	0·1952 0·2080	0·0128	0·0088	0·0222 0·0223	0·0001	**0·0089**
5	40	(*a*) 0·1284 (*b*) 0·1346	**0·0062**	0·2046 0·2150	0·0104	0·0064	0·0209 0·0232	0·0023	**0·0087**

Table III.—Cherry Laurel: area by disc method.

Assimilation experiment; August 29, 1907. Leaves detached, in glass case supplied with large excess of CO_2. Five hours of bright sunshine. A, fully insolated; B, shaded by double thickness of wet cotton cloth.

Numbers are grammes, and refer to the particular number of discs of leaf lamina.

Leaf.	No. of discs.	Dry weight.	Gain of dry weight.	CO_2 found on combustion.	Gain of CO_2.	Starch corresponding to gain of CO_2.	Ash found on combustion.	Gain of ash.	Calculated gain of dry weight.
A 2	14	(*a*) 0·1245 (*b*) 0·1316	**0·0071**	0·2192 0·2362	0·0170	0·0104	0·0085 0·0073	−0·0012	**0·0092**
3	15	(*a*) 0·1097 (*b*) 0·1119	**0·0022**	0·1919 0·2000	0·0081	0·0049	0·0062 0·0055	−0·0007	**0·0042**
4	15	(*a*) 0·1064 (*b*) 0·1162	**0·0098**	0·1861 0·2035	0·0174	0·0107	0·0066 0·0068	+0·0002	**0·0109**
B 2	15	(*a*) 0·1314 (*b*) 0·1286	**−0·0028**	0·2346 0·2246	−0·0100	0·0061	0·0067 0·0068	+0·0001	**−0·0060**
3	12	(*a*) 0·0861 (*b*) 0·0925	**0·0064**	0·1520 0·1655	0·0135	0·0083	0·0052 0·0050	−0·0002	**0·0081**
4	15	(*a*) 0·1267 (*b*) 0·1318	**0·0051**	0·2227 0·2304	0·0077	0·0047	0·0068 0·0075	+0·0007	**0·0054**

Analyses with Lead Chromate (to drive off CO_2 from ash).

Table IV.—Cherry Laurel: area by disc method.

Assimilation experiment; August 16, 1907. Leaves detached, in glass case supplied with large excess of CO_2. Time of exposure 11 A.M. to 6 P.M., 7 hours. Occasional sunny intervals, temperature in case varying from 18° to 27° C. according to illumination.

Numbers are grammes, and refer to the given number of discs.

Leaf.	Number of discs.	Dry weight.	Gain of dry weight.	CO_2 found on combustion.	Gain of CO_2.	Starch corresponding to gain of CO_2.
1	12	(*a*) 0·1070 (*b*) 0·1177	**0·0107**	0·1970 0·2126	0·0156	**0·0096**
2	12	(*a*) 0·0931 (*b*) 0·1042	**0·0111**	0·1682 0·1828	0·0146	**0·0090**
3	10	(*a*) 0·0805 (*b*) 0·0950	**0·0145**	0·1465 0·1701	0·0236	**0·0145**
4	9	(*a*) 0·0731 (*b*) 0·0784	**0·0053**	0·1302 0·1392	0·0090	**0·0055**
5	10	(*a*) 0·0866 (*b*) 0·0941	**0·0075**	0·2689 0·1717	0·0156	**0·0096**

Table V.—*Helianthus tuberosus*: area by disc method.

Assimilation experiment; August 19, 1907. Leaves detached, in greenhouse, under canvas. Time of exposure, 11.45 A.M. to 5.45 P.M. Intermittent sun. Temperature 23° C.

Number of discs.	Dry weight.	Gain of dry weight.	CO_2 found on combustion.	Gain of CO_2.	Starch corresponding to gain of CO_2.
65	(*a*) 0·1701 (*b*) 0·1923	**0·0222**	0·2689 0·3020	0·0331	**0·0203**

In Table VI two examples are given, in which asymmetry in dry weight and carbon content have been compared, to indicate the degree of accuracy obtained and the kind of experimental error to be allowed for in considering the results already given.

Table VI.—Cherry Laurel: Examples of Asymmetry.

All numbers are grammes, and refer to the actual area used.

a = left half-leaf. *b* = right half-leaf.

	Dry weight.	Ash.	Dry weight, less ash.	Percentage difference in organic substance.	CO_2 found on combustion.	Percentage difference in CO_2.
1	(*a*) 0·1044 (*b*) 0·1024	0·0051 0·0054	0·0993 0·0970	2·3	0·1927 0·1868	3·2
2	(*a*) 0·1541 (*b*) 0·1565	0·0080 0·0074	0·1461 0·1491	2·1	0·2783 0·2853	2·5

The differences in these percentages are of similar magnitude to the discrepancies in the previous tables, and it appears, therefore, that on the whole there is little in the results given in those tables to be explained otherwise than by errors incident to the technique of experiment. What differences there are, moreover, do not afford any explanation whatever of the large positive error found by Brown and Escombe in results obtained by the dry-weight method. The differences are small, and are for the most part in favour of the apparent gain of carbon. Indeed, in Tables I to III as a whole, the observed increase in dry weight is less than that calculated from the gain of carbon and ash. This might be due to the accumulation of substances containing a higher percentage of carbon than starch; or to some extent, especially with *Helianthus*, to shrinkage of the experimental half-leaf; for the leaf substance, as a whole, contains about 50 per cent. of carbon, whereas starch contains only 44 per cent.

Only in one instance is the difference too great to be easily explained (Table I, Leaf 2). Here it is possibly due to an exceptional experimental error.

Composition of the Gain of Dry Substance.

Some further points of interest connected with these analyses require consideration. In the first place, they suggest inorganic substance as a possible constituent of the gain of dry weight. It is not certain how far the observed changes in ash content were real, or how far only apparent and due to shrinkage; but differences of 12 or 13 per cent. (*cf.* Table II) are far too large to be reasonably accounted for by shrinkage. Moreover, an accumulation of inorganic substance is to be expected, at any rate in leaves attached to the plant and in detached leaves supplied with nutritive solutions.* It is also probable that these substances are translocated.

Broocks† and Menze‡ have also published ash analyses which tend to support these suggestions, although, again, the interpretation of their results is rendered somewhat doubtful by the possible occurrence of shrinkage. Hence, until more conclusive experiments have been carried out, it is necessary to bear in mind that changes of dry weight may consist in part of ash, perhaps to the extent of 5 per cent. or more, and in using Sachs' method it is advisable, for accurate work, to determine the ash content of the dried leaf material and deduct it from the dry weight.§

Having thus obtained the increase in organic substance, there still remains uncertainty as to its composition, and hence as to the amount of carbon dioxide which it represents.

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* The analyses in Table II suggest that even when detached leaves are supplied with distilled water an accumulation of mineral substance in the lamina may take place, presumably by translocation from the stalk and principal veins.

Menze's ash analyses may also indicate a similar translocation: he used detached leaves of trees like *Platanus*, *Tilia*, *Quercus*, exposed them to diffused light only, and protected them from wind by inverted beakers, so that it is more probable that there was some expansion than that much shrinkage took place. In the case of those leaves which were exposed in closed vessels of air free from carbon dioxide, the apparent fall in ash content observed was probably due to a positive area change.

† Broocks, W., "Über tägliche und stündliche Assimilation einiger Kulturpflanzen," 'Inaug.-Diss.,' Halle, 1892, pp. 17—20.

‡ Menze, O., "Zur Kenntniss der täglichen Assimilation der Kohlehydrate," 'Inaug.-Diss.,' Halle, 1887, pp. 10—12.

§ The ash content in Table III for Cherry Laurel shows irregular fluctuations; but the quantities dealt with were small, and the changes themselves scarcely exceed, in most cases, possible experimental errors. Cherry Laurel transpires but slowly, and a large increase would therefore not be expected.

The fact illustrated by the analyses, that the increase of dry weight corresponding to a given gain of carbon may be less than if it were all composed of starch, justifies the use, in this investigation, of the starch equivalent of the gain of carbon in preference to the carbohydrate equivalent as calculated by Brown and Escombe's "carbohydrate factor,"[*] which would have given a still higher value.

Brown and Escombe arrived at this factor, for reducing carbon dioxide absorbed to its equivalent gain of dry weight, from data in Brown and Morris' determinations of the relative amounts of starch and various sugars present in leaves of *Tropæolum majus* after vigorous assimilation. Besides leaving out of account that Brown and Morris did not determine the *increase* in sugars and starch in these experiments,[†] it does not allow for the probable accumulation of substances other than carbohydrates.

In particular there is evidence that proteid formation occurs in assimilating leaves.[‡] Saposchnikoff's researches[‡] are of especial interest in the present connection. He experimented with leaves assimilating normally, and, using the half-leaf method, determined the dry weight, carbohydrates, and proteid of the same portions of leaf material. His figures indicate that the increase in proteid may account for a considerable percentage of the increase of dry weight.

Since proteids contain a greater percentage of carbon than even starch, the total increase in dry weight must be less than if no proteid were formed at all. The "proteid factor" analogous to Brown and Escombe's carbohydrate factor (= 0·64) is about 0·54. Taking their carbohydrate factor for the carbohydrates themselves and supposing that the increase of proteid were equal to one-third of the increase in carbohydrates, as was the case in some of Saposchnikoff's experiments,[§] the true "dry-weight factor" would be 0·61, or practically equal to the "starch factor."

The true factor may vary considerably with conditions. One of Saposchnikoff's experiments[§] indicates that, given (1) a comparatively dull light so that the rate of formation of carbohydrates may not be great, and (2) an abundant supply of nitrates, practically the whole of the increase may take the form of proteid. In another experiment, in which distilled water was supplied to leaves instead of a nutritive solution, there was, if anything, a slight diminution in the amount of proteid present.*

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[*] *Loc. cit.*, p. 43.

[†] Brown and Morris, "Chemistry and Physiology of Foliage Leaves," 'Journ. Chem. Soc.,' vol. 63, 'Trans.,' 1893, p. 604.

[‡] *Cf.* Menze, *loc. cit.*; Crapowicki, "Eiweissbildung in den chlorophyllführenden Pflanzen," 'Bot. Cent.,' 1889, vol. 39, p. 536; Saposchnikoff, "Eiweissstoffe und Kohlehydrate der grünen Blätter als Assimilationsproducte," 'Bot. Cent.,' 1895, vol. 63, p. 246; Zaleski, "Eiweissbildung," 'Ber. d. D. Bot. Ges.,' vol. 15, 1897, p. 536; Palladin, "Influence de la Lumière sur la Formation des Matières protéiques actives et sur l'Énergie de la Respiration des Parties vertes des végétaux," 'Rev. gén. de bot.,' vol. 11, 1899, p. 81.

[§] *Loc. cit.*, p. 247.

The following table illustrates the possible range of variation in the weight of substance which might result from a given intake of carbon dioxide:—

Table giving Weights of various Substances which contain 12 grammes of Carbon, *i.e.* which are equivalent to 44 grammes of CO_2.

	Grammes.
Carbohydrates—	
Starch, $C_6H_{10}O_5$	27
Cane sugar Maltose Inulin } $C_{12}H_{22}O_{11}$	28·5
Hexoses, $C_6H_{12}O_6$	30
Ethereal oils, etc.—	
$C_{10}H_{16}$, $C_{10}H_{16}O$, etc.	14—15
Nitrogenous substances—	
Proteins	22—24
Amino acids—	
Asparagin	33
Leucin	21·8
Tyrosin	20·1

It appears from this table that the error involved in the assumption that the whole increase is of the average composition of starch might possibly be large. Fortunately, published experiments show that the substances which usually form the greater part of the increase are limited to those containing 40 to 50 per cent. of carbon, and that several of these are usually present, of differing composition, so that the average composition shown by the increase probably varies within narrower limits. Taking this smaller series of products, the weight corresponding to 12 grammes of carbon varies between 24 and 30 grammes. This means an extreme error, if the "*starch* factor" is used, of ± 3 grammes in 27 grammes, or about 10 per cent.

Variations of this kind may occur in the same plant under different conditions, and certainly occur in different plants, so that an error is involved in comparative investigations of all kinds, and the results will therefore only be approximate. But, as the analyses show, there is sufficient correspondence between the increase in dry weight and the starch equivalent of the gain of carbon to enable a good deal of wide comparative work with varying plants and conditions to be done by the dry-weight method without much vitiation by such errors of interpretation.

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* *Loc. cit.*, p. 247. Since, in assimilation experiments, detached leaves are usually supplied with distilled water only, a decrease in proteid might be expected from this experiment of Saposchnikoff. The composition of the increase would thus be different for attached and detached leaves.

For more exact work a convenient and rapid method of carbon analysis would be a distinct advantage. The wet-combustion method described by Hall, Miller, and Marmu,[*] depending on the absorption and estimation of carbon dioxide by the method elaborated by Brown and Escombe,[†] has been suggested to me by Dr. F. F. Blackman as possibly adaptable to the purpose. Besides rapidity and convenience, it would have the advantage that the whole of the carbon is driven off from the ash as well as from the organic material, and ash determinations would be avoided. However, the possibility of using this method has still to receive further consideration, especially in respect to the small quantity of material which can be analysed, owing to the limited amount of carbon dioxide that a Reiset tower will efficiently absorb. It must suffice here to point out that along some such lines modifications may be possible which would avoid all the errors of interpretation that have their origin in differences of composition of the photosynthetic products.

Section IV.—The Error from Lack of Symmetry.

This source of error, which Brown and Escombe conclude to be serious, Sachs[‡] apparently assumed to be negligible when dealing with corresponding patches on either side of the midrib of a leaf.

He realised, however, the necessity of taking precautions to ensure as great a degree of similarity as possible between the pieces of material to be compared. The same templets were used on the two sides of the leaf, and care was taken to include as nearly as possible the same proportion of veins in corresponding pieces, and where possible to avoid outstanding veins altogether. The leaves were also carefully selected for their freedom from blemishes, such as dry spots or crumpled areas.

Sachs also used a number of leaves for each experiment, and so reduced the probable error from asymmetry, though he does not make a point of this.

Menze, experimenting with comparatively small leaves, had to use the greater part of each half-leaf, and could not select similarly veined portions.

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[*] "The Estimation of Carbon in Soils and Kindred Substances," 'Journ. Chem. Soc.,' vol. 89, 'Trans.,' 1906, p. 595.

[†] 'Phil. Trans.,' B, vol. 193, 1900, p. 289.

[‡] *Loc. cit.*

He was led in consequence to take particular notice of apparent symmetry, and concluded that it was impossible to find completely symmetrical leaves. Not only was dissimilar distribution of veins sometimes impossible to avoid, but differences in thickness were observed; and these could not be taken into account in selecting leaves for experiment.* Nevertheless he made no measurements of the extent to which such differences could affect his results.

When Brown and Morris† made the first actual determination of the effect of such asymmetry, the difference was found to be considerable, and of a higher order of magnitude than the errors of weighing and of measuring area, the only errors mentioned and estimated by Sachs. They aimed at repeating Sachs' procedure as closely as possible, and, using seven leaves of *Helianthus annuus*, found a difference of 0·43 gramme per square metre (*i.e.* 1·1 per cent.) between the portions from right and left halves respectively. Great care was taken in this experiment to choose pieces as similarly veined as possible, which was made more easy by the use of glass templets.

If fewer leaves and smaller areas were employed the error could be considerably greater than that found by Brown and Morris using seven leaves and 800 square centimetres. Brown and Escombe's results for single leaves illustrate this. One of their *Catalpa* leaves showed a difference of nearly 6 per cent. Their results may be quoted here for comparison with others to be given later.

Brown and Escombe's Asymmetry Determinations.‡

	Percentage difference.	
Catalpa bignonioides	−3·9	
	−4·3	
	+2·3	
	−5·7	
	−0·7	
Average	±3·4	
Catalpa purpurea	+2·5	[misprinted +2·0]
Catalpa Bungei	+1·4	
	−2·3	[,, −1·7]
Tropæolum majus	+0·4	
Polygonum Weyrichii	−1·1	[,, −0·1]
Mean of all the results..............	±2·4	[,, ±2·2]

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* *Loc. cit.*, p. 12.

† *Loc. cit.*, p. 625.

‡ *Loc. cit.*, p. 60, Table IX.

In these determinations the area of the whole of each half-leaf was measured by the planimeter method. A number of prominent veins were therefore included, and careful examination of a number of *Catalpa* leaves, even if chosen for their apparent symmetry, reveals the fact that the venation is not symmetrical. It seemed unlikely that the mesophyll itself would exhibit differences as great as those found by Brown and Escombe for mesophyll *plus* veins. For this reason modifications in the methods of area determination have been adopted* with the object of avoiding all outstanding veins. The rotating punch (p. 42) is very useful in this respect, owing to the small size of the discs it will accurately cut, although it is not so rapid in use as templets. In certain cases small templets have also been used.

The advantage of avoiding veins altogether is clearly shown by some of the results given in the tables that follow. For instance, the average difference for 11 leaves of *Helianthus* when the punch method was used (Table VIII) was ± 1·4 per cent., whereas four leaves for which the templet method was used, although the main veins were avoided, showed the considerably higher average difference of ± 2 per cent.†

The experiment with *Paulownia imperialis* (Table XIII) gave results still more striking. The large leaves of this tree are strongly veined, but between the main veins are areas practically without projecting veins. From these areas pieces can be cut with templets as small as 4 cm. by 2·5 cm., with considerable accuracy. Towards the margin, especially near the base, are other areas traversed by the slightly projecting ultimate branches of main veins, but these are nevertheless not prominent enough to increase appreciably the errors of cutting. The contrast between the differences shown by pieces including veins, and by other pieces from the "veinless" areas, is instructive. For the latter the average difference was ± 1·4 per cent., for the former ± 5·9 per cent., more than four times as much.

The results for *Catalpa bignonioides* (Table XIV), obtained by the templet method without avoiding veins, correspond fairly well with Brown and Escombe's results for the same plant.‡ On the whole, omitting leaf 5, where the extreme difference was due to the slight convexity of one half, the differences are rather less than theirs.

The other tables give the results of experiments with other leaves.

The general plan of dealing with individual leaves has been followed in order to determine how far the different results given by individual leaves in the same assimilation experiment were to be accounted for by asymmetry, and how far other sources of error were to be reckoned with. The leaves used were of several different types: Cherry Laurel, thick and somewhat leathery; *Helianthus tuberosus*, thin but coarse, and *Tropæolum majus*, thin and delicate, both without a strongly supporting venation; Lime and *Cercis*, similarly contrasted as regards delicacy but with a very efficient supporting network of veins. The templet, planimeter, and punch methods were all used in determining areas.

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* See Section VI, pp. 39—44.

† Errors involved in area determination by templets may account for part of this difference. *Cf.* Section VI, pp. 39, etc.

‡ Quoted on p. 13.

Attention has been concentrated on *Helianthus tuberosus* and the Cherry Laurel, since they were also used for assimilation experiments.

TABLES SHOWING DEGREE OF ASYMMETRY EXISTING IN VARIOUS LEAVES.

Explanatory Notes.

The differences given were obtained by subtracting the dry weight of the right half-leaf from that of the left, and the percentages have been calculated in terms of the latter. The percentage differences are given in column 2, the actual differences in milligrammes per square decimetre in column 1.

In each table, either the range of areas used in the series of experiments is given at the head of the table, or the actual area cut from each half-leaf is given, in a special column, for each experiment. Where the rotating punch* was used, the number of discs is given instead of the area. The area of each disc was 0·804 square centimetre.

In some cases the number of discs cut from each side was not the same: this was due to the slightly unequal area of the two sides, or to different arrangement of the veins, which were avoided where possible. For instance, in using the disc method with leaves of *Helianthus tuberosus*, care was taken to make the discs equally representative of the mesophyll on either side, whereas "symmetrical" cutting relative to the midrib would have been impossible. This applies also to some of the experiments with Cherry Laurel. The lateral veins in the larger leaves were sufficiently distant from one another to allow of discs being cut from between them: absence of perfectly symmetrical placing of the veins led to the cutting of different numbers of discs in such cases. Later, the supply of large leaves was exhausted, and the smaller leaves had less prominent veins, so that there was less objection to including them. Discs were then cut in equal numbers and from symmetrical positions from the two sides.

Table VII.—Cherry Laurel. Cut by rotating punch.

Number of discs from each half-leaf varied with size of leaf from 10 to 25.

	(1)	(2)
	milligrammes.	per cent.
Single leaves—Experiment 1	−12	−1·3
,, 2	−25	−2·8
,, 3	−16	−2·0
,, 4	+27	+2·5
,, 5	+ 1	Nil
,, 6	−25	−3·0
,, 7	− 8	−1·1
,, 8	+26	+3·3
Averages of 1—8	±17	±2·0
Leaves 1—8 taken together	− 4	−0·5
Several leaves taken together—		
8 leaves—Experiment 9	− 1·9	−0·2
6 ,, ,, 10	−33	−2·7
7 ,, ,, 11	+17	+1·5
6 ,, ,, 12	+17	+1·4

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* See p. 42.

Table VIII.—*Helianthus tuberosus.*

A.—Using the rotating punch: discs cut from between outstanding veins, 20 to 42 from each half-leaf. In the experiments with single leaves, the percentage differences are given to the nearest 0·5.

	(1)	(2)
	milligrammes.	per cent.
Single leaves—Experiment 1	Nil	Nil
„ 2	−7	−3·5
„ 3	Nil	Nil
„ 4	−1	−0·5
„ 5	+1	+0·5
„ 6	−6	−2·5
„ 7	+9	+3·0
„ 8	−4	−1·5
„ 9	+5	+2·0
„ 10	−1	−0·5
„ 11	+2	+1·0
Averages of 1—11	±3	±1·4
Leaves 1—11 taken together	Nil	Nil
4 small leaves taken together—Experiment 12	+1·9	+0.7

Table IX.—*Helianthus tuberosus.*

B.—By templet method: total area cut from each half-leaf given for each experiment. It was not recorded which dry weights correspond to right and left halves respectively.

		(1)	(2)
	sq. cm.	milligrammes.	per cent.
Single leaves—Experiment 1	20	8·5	2·6
„ 2	70	8·7	2·6
„ 3	20	5·5	1·6
„ 4	40	4·0	1·4
Averages of 1—4	...	±6·7	±2·0

Table X.—*Tropæolum majus.*

A. Experiments 1—4. Area of entire half-leaves by planimeter method: areas between 47 and 64 sq. cm.

B. Experiment 5. By rotating punch: 50 discs from one half (whether left or right not recorded), 54 from the other; differing number due to different distribution of veins on the two half-leaves.

	(1)	(2)
	milligrammes.	per cent.
Single leaves—Experiment 1	+ 9	+3·8
„ 2	− 6	−2·1
„ 3	+12	+4·0
„ 4	+ 4	+1·5
„ 5	12	4·8
Averages of 1—5	± 9	±3·2

Table XI.—Lime.

A. Experiments 1 and 2. By planimeter method : area about 30 sq. cm.
B. Experiments 3 and 4. By rotating punch. Experiment 3, 10 discs ; Experiment 4, 13 discs.

	(1)	(2)
	milligrammes.	per cent.
Single leaves—Experiment 1	−13	−3·3
„ 2	+28	+4·4
„ 3	+15	+4·0
„ 4	+ 4	+1·0

Table XII.—*Cercis.* By rotating punch.

Number of discs 17—20.

	(1)	(2)
	milligrammes.	per cent.
Single leaves—Experiment 1	+15	+3·5
„ 2	+ 9	+2·5

Further Experiments on Asymmetry.

The experiments in Tables XIII and XIV were performed in connection with assimilation experiments. The leaves were cut from trees in the University Botanic Garden in the early morning, while they were still wet with dew, were carried to the laboratory, wrapped in a damp cloth in a vasculum, and used at once.

Templets were used in all the experiments.

Table XIII.—*Paulownia imperialis.*

Three leaves furnished material for six experiments ; in four of these, only pieces without prominent veins were cut, in the other two the pieces included minor outstanding veins.

The area is given for each experiment. Where two pieces were cut from each half-leaf for a single experiment, the areas of the individual pieces are connected by a plus sign.

Leaf.	Portion.		(1)	(2)
		Avoiding veins.		
		sq. cm.	milligrammes.	per cent.
1	1	30	+ 9·7	+1·5
1	2	10+10	+ 9·5	+1·6
2	1	21+10	− 6·1	−0·9
3	1	21+10	+ 9·7	+1·6
		Including veins.		
2	2	21+15	−39·7	−5·9
3	2	20	−33·5	−6·0

	Avoiding veins.	Including veins.
	milligrammes.	milligrammes.
(1) Average difference per square decimetre ...	±9	−37
Difference per square decimetre taking all together	+5	−37
(2) Average percentage difference	±1·4	± 5·9
Percentage difference taking all together ...	+0·8	− 5·9

Table XIV.—*Catalpa bignonioides.*

Except in the case of Leaf 1, the midrib was removed before cutting pieces from the half-leaves with the templet: area in each case 50 sq. cm.

Leaf.	(1)	(2)
	milligrammes.	per cent.
1	+19·0	+4·0
2	− 1·6	−0·3
3	+10·6	+1·7
4	−10·6	−1·8
5	+38·8	+8·1
6	−17·8	−3·1
The left half of Leaf 5 was seen to be not perfectly flat; the results for this leaf have therefore been omitted from the following averages.		
Excluding Leaf 5.		
Average	±11·9	±2·2
Taking all together	− 0·1	Nil

The results as a whole prove the necessity for determining this error for each plant used in an investigation. Asymmetry, to the extent of an average of 1½ to 3 per cent., is shown by all the leaves examined, and in extreme cases it varies from zero to 4 per cent. or more. *Catalpa bignonioides* shows a higher degree of asymmetry than the average.

The seriousness of this asymmetry in assimilation experiments is greater in thick than in thin leaves. Thus in Cherry Laurel, in an experiment lasting seven hours, asymmetry to the extent of 2 per cent. will mean an error of about ± 3 milligrammes per square decimetre (± 0·3 gramme per square metre) per hour, while in *Helianthus tuberosus* it will be less than ± 1 milligramme per square decimetre per hour. For a rate of increase of 10 milligrammes per square decimetre per hour, such as Brown and Morris found for detached leaves of *Helianthus annuus*, the average percentage error would be 30 per cent. in Cherry Laurel, 10 per cent. in *Helianthus.*

By using a number of leaves this error may be reduced roughly in the inverse ratio of the square root of the number of leaves used. In Brown and Morris's example, for instance, the asymmetry of seven leaves of *Helianthus annuus* taken together was 1·1 per cent. Some similar results for *Helianthus tuberosus* and Cherry Laurel will be found in the tables, though with leaves of the latter the error may still be high (*e.g.* 2·7 per cent. for six leaves in Experiment 10). Further discussion of these results will be postponed till the errors as a whole are discussed.[*]

Attention may be called here to the fact that all asymmetry determinations are affected by errors classed above as errors of technique; but, as will be shown later,[†] these are as a rule relatively small, and account for only a small part of the differences shown in the tables.

Section V.—ON CHANGE OF AREA DURING EXPERIMENT.

Brown and Escombe's conclusion, that the results given by the dry-weight method are too high, was based not only upon their direct test with Catalpa, but also on a consideration of the general high level of the results obtained by themselves and others when using it. In particular, the value found by Sachs for the rate of assimilation in detached leaves of *Helianthus annuus* was far higher than any which they observed in their experimental chamber.[‡]

If the method itself is really responsible for these high values, some change, related in a definite way to the conditions of experiment, must take place in the experimental half-leaf.

Of such possible changes, that of water fixation suggested by Brown and Escombe has been disposed of in Section III, where it is shown that Sachs' results would have been approximately the same had he measured *carbon content* instead of dry weight, all other details of his method remaining the same.

On the other hand, shrinkage in area due to loss of water could produce errors consistently in a positive direction, for the conditions under which a leaf might be expected to assimilate most rapidly are just those conditions which are favourable to increased evaporation.

For instance, Broocks[§] observed a much greater increase of dry weight in fully insolated leaves of the Sugar Beet than in leaves shaded from direct sunlight. If the more rapid evaporation to be expected in the sun resulted in appreciable shrinkage, the whole or part of this difference might be only apparent: it is, in fact, uncertain whether either intensity of light or leaf temperature were limiting factors to assimilation in these experiments of Broocks.

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[*] Section VIII, p. 48.

[†] Sections VI and VII.

[‡] Sachs' result was 16 milligrammes per square decimetre per hour (*loc. cit.*, p. 25). The highest rate found by Brown and Escombe for *H. annuus* was 5·5 milligrammes (*loc. cit.*, p. 44).

[§] *Loc. cit.*, p. 17.

Again, the conditions under which dry-weight assimilation experiments have usually been conducted would tend to exaggerate errors from shrinkage. In order to ensure that the leaves are starch-free to begin with, they are covered with tinfoil overnight, or else the experiment is commenced soon after sunrise. In either case the leaf is likely to be in a condition of maximum turgidity at the beginning of the experiment. On the one hand, close confinement prevents the escape of moisture, often to such an extent that water vapour condenses on the inner side of the tinfoil; while on the other hand the relatively low temperature and high degree of humidity prevailing at night and in the early morning are also just the conditions to retard evaporation. Thus the area of the first half-leaf is measured, or pieces cut from it, immediately after treatment calculated to produce full turgidity. The other half is exposed to direct sunlight, or, even if the conditions are not so extreme as this, to conditions favourable to considerable evaporation. Hence the turgor might reasonably be expected to diminish and with it the area, and a positive error to be thus introduced.

Moreover, the frequent mention by previous workers of the difficulty of avoiding the wilting of detached leaves suggests that the much greater increase in dry weight shown in experiments with detached leaves might, in part at least, be only apparent. Even the results of translocation experiments would be too great, for the conditions under which these experiments are carried out are favourable to increase in turgidity, which would result in an apparent decrease in dry weight per unit of area and an over-estimate of the amount translocated. In addition to this, the leaves may still be slowly growing, and their growth expansion would introduce a further error in the same direction.

Sachs was aware of the danger of comparing turgid with flaccid leaves, and in his experiment with detached leaves of *Helianthus annuus* he floated the experimental halves on water for half an hour at the end of the experiment to make them turgescent. Yet neither Sachs, nor any subsequent experimenter, until Brown and Escombe published their results, gave any evidence of having tested whether shrinkage affected his own experiments appreciably; nor, it may be added, of having ascertained that the leaves used had ceased growing. They assumed that, so long as the condition of a leaf appeared to the eye to be approximately the same, such changes could not be great enough to affect their results to an appreciable extent.

The following experiment shows how unjustifiable this assumption was:—

A leaf of *Helianthus tuberosus* was allowed to dry up slowly, and at several stages in the process records were made of its weight, apparent degree of turgidity, and linear dimensions.

The turgidity records are shown in fig. 2. They are rough diagrams indicating the positions assumed by the leaf when its stalk was placed (1) vertically, and (2) horizontally. Four stages are shown (A, B, C, D), beginning with Stage A, which represents the condition of the leaf after floating on water under a bell-jar till fully turgid.

The linear measurements were made with a millimetre scale between crosses marked on the leaf with waterproof ink. Fig. 1 shows the disposition of the marks, and of the lengths measured.

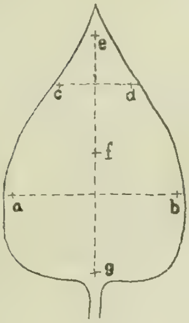

Fig. 1.

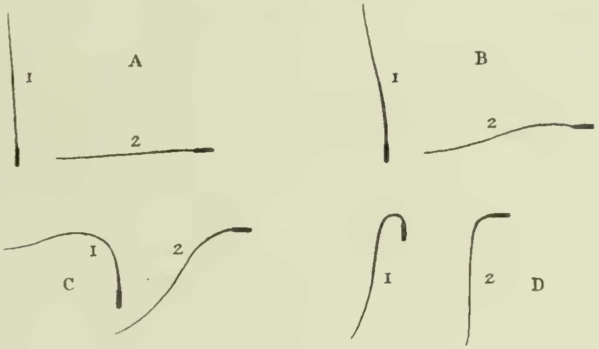

Fig. 2.

In Table XV the records of weight and dimensions are given, for the stages indicated by the letters in the first column, in the order in which they were made. Stage A was twice returned to by floating the leaf on water.

The *changes* in the weight and dimensions from stage to stage are given in Table XVI, as percentages of their value at Stage A.

Table XV.

Lengths are given to nearest $\frac{1}{4}$ mm.

Turgidity diagram.	Dimensions in centimetres.				Fresh weight in grammes.
	a—b.	*c—d.*	*e—f.*	*f—g.*	
A	8·00	5·45	9·45	7·70	2·56
D	7·65	5·20	8·90	7·60	1·97
A	8·00	5·45	9·47	7·70	2·56
B	7·77	5·30	9·30	7·67	2·26
C	7·70	5·25	9·20	7·65	2·06
A	8·05	5·45	9·47	7·72	2·67

Table XVI.

Changes of dimensions from stage to stage as percentages of dimensions at Stage A.

	a—b.	*c—d.*	*e—f.*	*f—g.*	Decrease in area: estimated.	Loss of weight.
A—B	2·9	2·8	1·6	0·4	4	11·7
B—C	0·9	0·9	1·1	0·3	1·5	7·8
C—D	0·6	0·9	3·2	0·7	2·5	3·5
A—D	4·4	4·6	5·9	1·4	8	23·0

As far as external appearance went, Stage B was not readily distinguishable from Stage A, yet in passing from A to B the leaf had lost over 10 per cent. of its weight by evaporation, and had diminished in area by about 4 per cent. Passing on to C, a condition occurring quite commonly on the plant in the open air, there was a further loss of water of 8 per cent., and a much smaller change in area of about 1·5 per cent. From C to D the loss of water of only 3·5 per cent of the original fresh weight was accompanied by a rather greater loss of area of about 2·5 per cent., due to a greater shrinkage in length at this stage, and this brought the leaf to what may be described as a condition of complete flaccidity, such as occurs occasionally in hot sunny weather.

Summing up the changes, we find that between maximum turgidity and complete flaccidity the leaf decreased in weight by 23 per cent. through loss of water, and shrank by about 8 per cent. of its area.

Change of Area under Natural Conditions.—Helianthus annuus.

A large number of observations of a similar character have been made which show that the liability to shrinkage varies from leaf to leaf, with the character of the mesophyll itself, and with the nature of the epidermis and of the supporting venation. Most interesting of all is a series of observations on *Helianthus annuus* made during the period of fine weather at the end of July and beginning of August of 1908.

This plant is of especial interest, because it was with its leaves that Sachs obtained some of his highest values for the rate of assimilation. He commenced his experiment with *attached* leaves at 5 A.M., and continued it till 3 P.M., and my original object was to determine by direct measurement the change of dimensions which takes place between those hours on a bright sunny day.

Although the observations were begun soon after the ground had been soaked with heavy rain, the amount of shrinkage in full sunlight was astonishing. Even more surprising was the sensitiveness of Sunflower leaves to changes in the intensity of the sunlight. In the following pages is given a selection of the results, which are of interest apart from the immediate purpose for which they were obtained.

Lengths were marked off on the leaves with fine black crosses over areas carefully selected for their flatness. They were measured with a millimetre scale: a support below the leaf, consisting of a flat rule covered with plush, yielded to the outstanding veins and allowed the upper surface of the leaf to remain flat under the scale.

Since the distances measured were of the order of 10 cm., a change of dimensions of 1 per cent. was easily detected.

Measurements were taken both transversely and along the midrib. With a good flat leaf four measurements were made (as in Leaves I and V, in the following tables and diagrams), one across each half at its widest part, another right across the leaf nearer the tip, and a fourth along the midrib.

The percentage change of area was estimated by averaging the percentage changes in the cross measurements, and adding the percentage longitudinal change.

Most of the following observations were made on three plants growing in the University Botanic Garden. Six leaves were chosen, and are referred to as I to VI respectively. I, III, and IV were on the same plant and II on another plant in the same clump, in a fairly exposed situation. V and VI were on a plant in the north border, sheltered from the wind.

The curves in fig. 3 represent the percentage changes which took place in the area of Leaves I, II, and V over a period of seven days. The measurements were begun on July 22, at 5 A.M., the dimensions obtained on this occasion were taken as standards, and the variations subsequently observed were calculated as percentages of those standard dimensions. The percentage changes of area, estimated as described above, are plotted as ordinates against the times as abscissæ.

Examining first the curves* for July 22, which was a bright sunny day throughout, the remarkable fall in the curve for Leaf I, between 5 A.M. and noon, is very striking, and indicates a shrinkage in area of 5 per cent. Recovery was rather less rapid, and by 3 P.M. the area was still 3·2 per cent. less than at 5 A.M. The appearance of the leaf gave evident indications of a decrease in turgidity by waving of the lamina, which was not so fully extended as when quite turgid at 5 A.M. In addition to this the stalk curved over, so that the leaf hung downwards. Unless signs of relative flaccidity were being specially looked for, the position of the leaf might perhaps have been attributed to heliotropic curvature, and the waving of the lamina to its pendant position. In the early morning clear heliotropic movements were noted.

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* It is scarcely necessary to remark that the curves themselves are only of rough pictorial value, and do not accurately indicate the actual changes that take place in the periods intervening between the actual measurements.

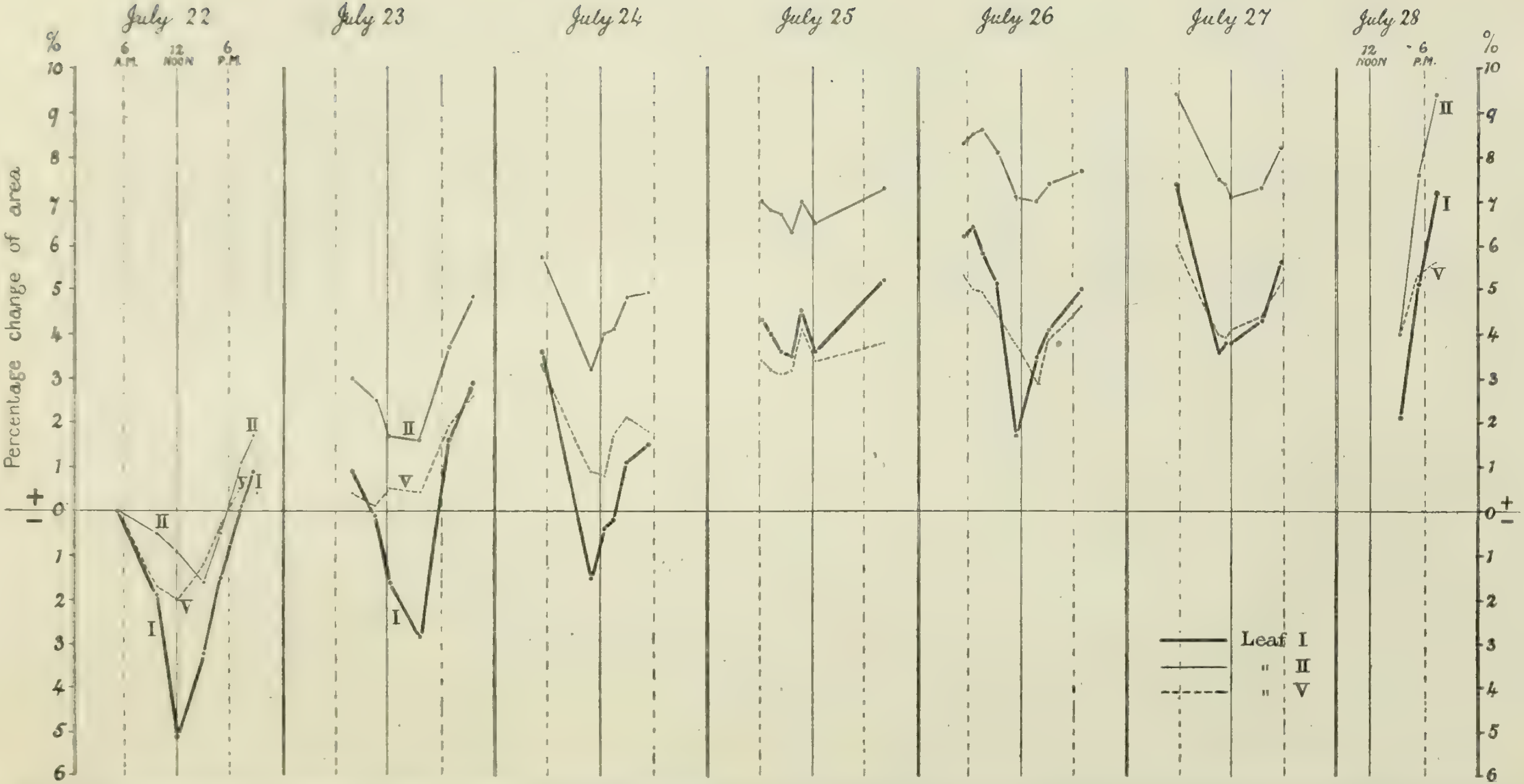

FIG. 3.—Curves showing changes of area of three leaves of *Helianthus annuus* during July 22 to 28, 1908. The ordinates are percentage differences from the area at 5 A.M. on July 22.

Leaves III and IV on the same plant (results not plotted) behaved in a similar way. Leaf II, on the other hand, appeared fully expanded and turgid right on till July 28, after a very trying windy morning. Even then so slight were the visible signs of change that, unless they had been very carefully watched for, the leaf would undoubtedly have been described as fairly turgid. Nevertheless, between 3 P.M. and 6 P.M., during its recovery, the increase in area was 5·4 per cent., and from 5 A.M. to 3 P.M. the shrinkage must have been at least 6 per cent.

The good condition of Leaf II was partly due to its sturdier construction, but later observations have shown that the ability to resist extreme conditions is largely a function of the age of the leaf. It was observed on August 7, under by no means extreme conditions, that Leaf I, which was situated fairly low on the plant, was completely flaccid, the lamina hanging in folds against the midrib. Higher up on the same plant, Leaves III and IV were only slightly waved, and towards the top of the plant were leaves which appeared completely turgid.

It was also observed that Leaf I became less and less able to withstand the heat of the sun, and although on July 22 it was only slightly waved at midday, yet on August 7, under conditions which were if anything less severe, it reached the completely limp state just described. The same phenomenon was even more strikingly shown by Leaf II, which up to July 27 had not shown the slightest signs of flaccidity to the eye. After the wind and sun of the 28th it quickly deteriorated and became early in August as unable as Leaf I to withstand bright sunshine without collapsing.

Referring for a moment to Leaf 5 it may be remarked how much less shrinkage this leaf shows than Leaf I. The curves for this leaf are much more comparable with those for Leaf II. This is to be attributed in part to similarity of age: in part, as the curves for July 28 show, to the more sheltered situation of Leaf V, in which it was protected on that day from the wind that continually agitated the plants in the open bed.

Fig. 4 shows details of all six leaves for a day of very varying illumination, haze and sun alternating as stated in the figure. Corresponding with these alternating periods of hazy and brighter weather, the slope of the curves for Leaves I and II changes from steep to nearly horizontal, indicating less rapid recovery in brilliant illumination. It will be observed that while Leaves III and IV show a similar alternation, Leaves V and VI behave differently, though they agree very closely indeed with each other. This difference is doubtless to be attributed to disturbing factors peculiar to the situation, but the records have not afforded any clue to the nature of those factors.

The rise in the curves for the following day, July 25 (fig. 5), shows in a very striking way the effect of clouds and rain. At 9.30 the sky was covered with thin grey cloud, through which the sun was only faintly visible, and from 10.15 to 10.35 a light shower of rain fell. During this short period of little over an hour the leaves had all expanded to the extent of 0·7 to 1 per cent., and in the brighter interval which followed again decreased in area by nearly the same amount.

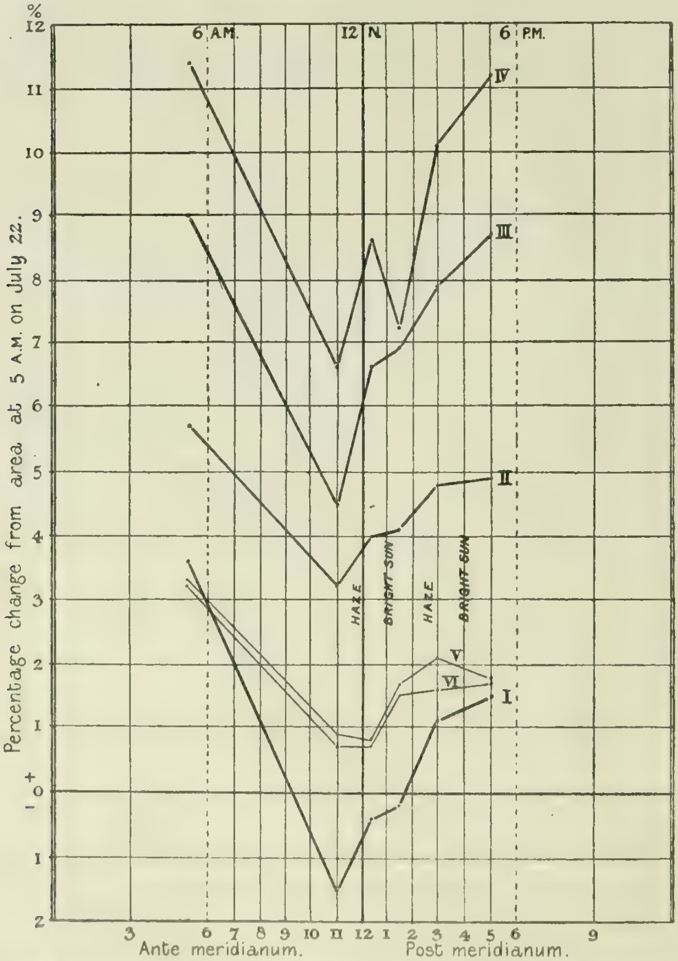

FIG. 4. Curves showing more rapid expansion of leaves of *Helianthus annuus* when haze diminished the intensity of the sunlight. Bright sunshine prevailed until 11 A.M., two hazy periods occurred between 11 A.M. and 3 P.M., separated by an interval of bright sunshine lasting from 12.30 to 1.30 P.M., July 24, 1908. Ordinates are the percentage excess of the area over that at 5 A.M. on July 22.

These observations were followed up by a series of measurements made on a single leaf (Leaf III) between 10 A.M. and 12.20 P.M. on August 1, for the most part every 5 minutes. The curves (fig. 6) show the percentage changes in the linear dimensions, the continuous and the broken lighter lines corresponding respectively to transverse measurements of the left and right halves of the leaf, and the heavy line to measurements along the midrib. The sky was somewhat cloudy, and periods in which clouds passed over the sun are indicated by shading on the right-hand side, corresponding to the

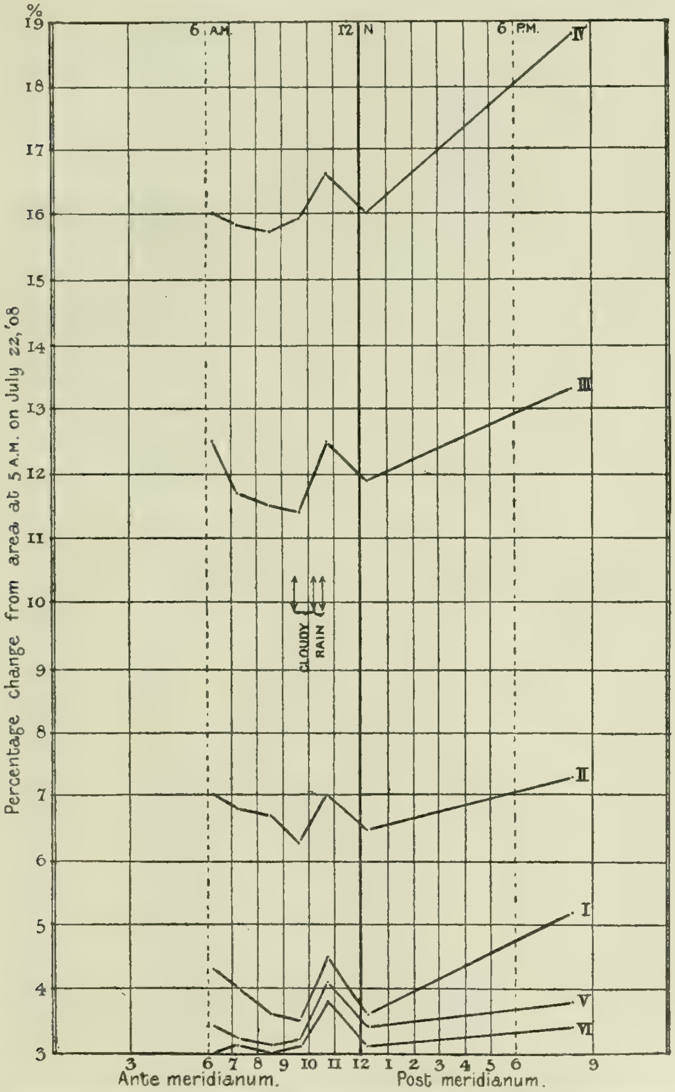

FIG. 5.—Curves showing temporary expansion of leaves of *Helianthus annuus* during interval of cloud and rain on July 25, 1908. Ordinates are the percentage excess of the area over that at 5 A.M. on July 22.

times which are shown on the left. The 5-minute observations commenced at 10.45, and the changes observed, as bright sunshine alternated with shade, were remarkably rapid. For instance, the left half of the leaf increased

1·6 per cent. in width in the 5 minutes elapsing between the measurements made at 10.50 and 10.55. It is highly probable that with more accurate and

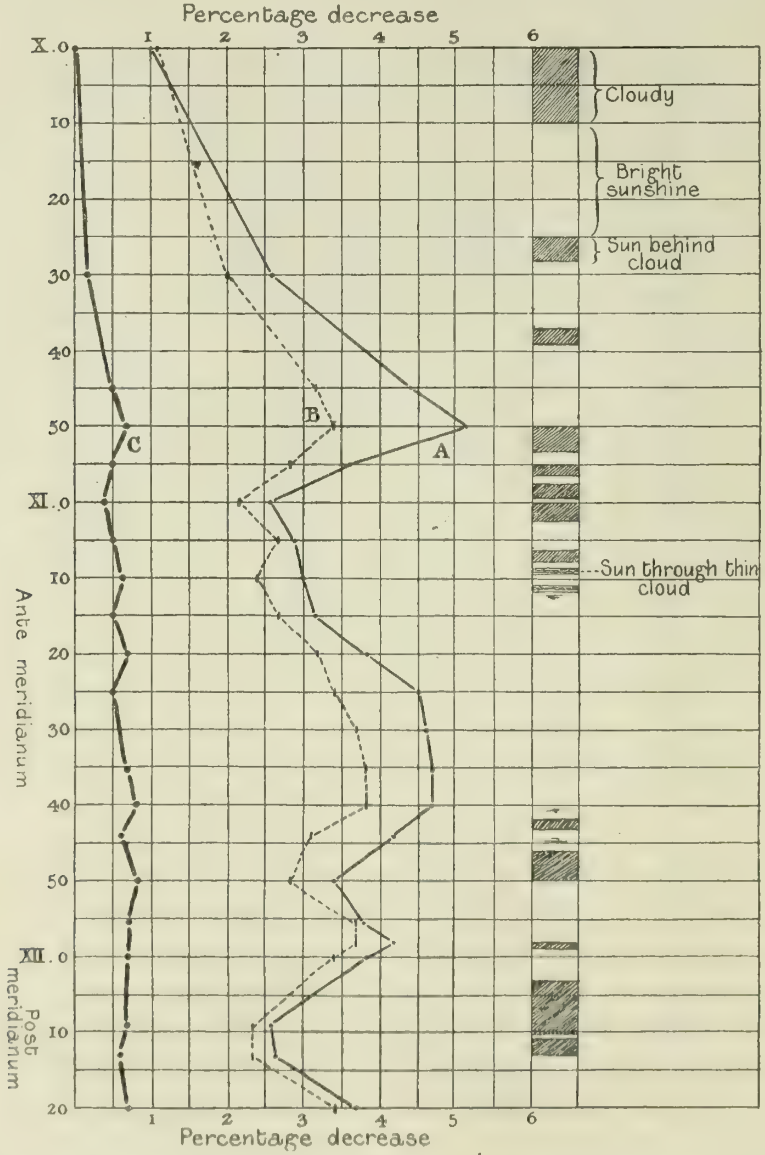

Fig. 6. Curves showing the rapidity of reaction of a leaf of *Helianthus annuus* to varying illumination, on August 1, 1908.

Abscissæ are percentage differences from the linear dimensions in the early morning of the previous day. Curves A, B, C, refer respectively to measurements across the left and right half-leaves, and a measurement along the midrib.

continuous observations still greater sensitiveness and rapidity of reaction to changes of illumination would have been revealed.

It may be pointed out that the two halves of a leaf are not symmetrical even with respect to their change of dimensions with change of conditions. For example, between 10 and 10.50 A.M. the width of the left half of the leaf had shrunk 4·2 per cent., while the width of the right half at the same level had only decreased by 3·3 per cent. Similar differences were observed while making the measurements from which the curves on fig. 3 were constructed. It was also noticed that the leaf shrinks more and sooner, and recovers more tardily, towards the tip than it does nearer the base, a fact which is doubtless to be explained by relative distance from the water supply. As was to be expected from the nature of the tissues involved, the variations along the midrib are comparatively small, but, as shown also on fig. 6, correspond closely in sign with the variations in transverse dimensions. One other point is worthy of note. The right half of Leaf I showed a faster rate of growth from day to day than did the left half, as well as, if anything, a slightly smaller amount of shrinkage. With the possibility established of such differences as these between two sides of a leaf, a certain degree of asymmetry in the dry weight of the mesophyll itself is no longer surprising, although it does not necessarily follow that true growth expansion involves an alteration in dry weight per unit of area under comparable conditions of turgidity.

Fig. 7 shows the results of observations on Leaves 7, 8, and 9, belonging to plants growing on the south side of the University Botany School. The curve for Leaf I on the

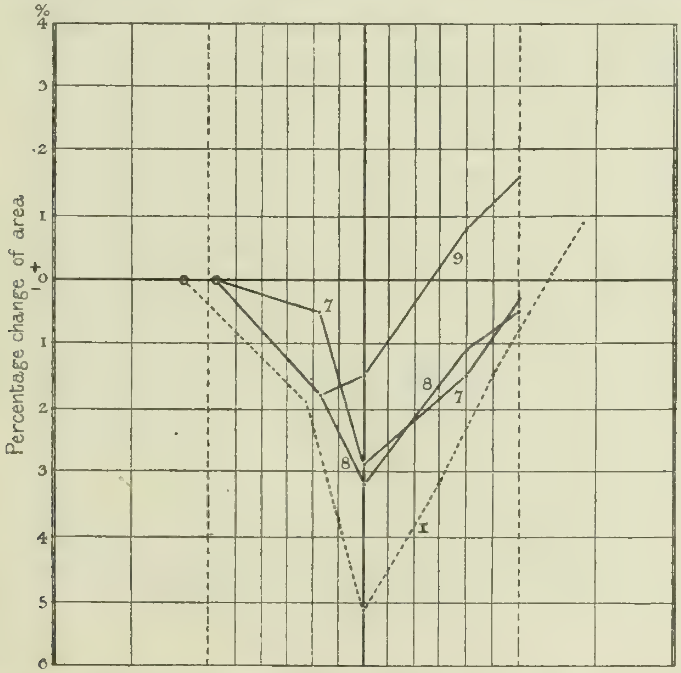

FIG. 7.—Shrinkage in area and recovery of leaves of *Helianthus annuus* on July 22, 1908. 7, 8, and 9, behind Botany School. I in open bed in Botanic Garden, for comparison. Leaves 8 and 9 were in the shade of lime trees till 9 A.M.; Leaf 7 till 11 A.M.

same day is given for comparison. The chief point of interest is the contrast between Leaf 7 and Leaves 8 and 9 at 10.15 A.M. The shadow of an avenue of limes left the latter about 9 A.M., so that by 10.15 they had been in full sunlight for more than an hour. Leaf 7, on the other hand, belonged to a plant several yards nearer to the trees, and was not insolated till 11 A.M. Consequently by 10.15 the latter had decreased in area very little, only 0·5 per cent., while Leaves 8 and 9 show a decrease by the same time of 1·8 per cent.

Sachs' Experiments and Shrinkage Errors.

The extreme importance of shrinkage as a source of error in the dry-weight method is fully demonstrated by these results. To show the relative magnitude of the errors which are possible, it will be convenient to take the experiments described by Sachs and estimate the errors that shrinkage may have introduced into his results.

There is much difficulty in selecting, from my measurements of shrinkage, results which can fairly be compared with Sachs' experiments, for there is no means of knowing to what extent he took notice of differences in the appearance of the leaves he used. One hesitates to put a high value to the personal equation of an experimenter of Sachs' experience and ability; although, on the other hand, after an interval of 10 hours, slight differences are easily overlooked, and even half-consciously ignored when the extent of their effect on the experiment in hand is not suspected. What follows is therefore not to be regarded as finally condemning Sachs' original results, however much doubt it may throw on their trustworthiness.

He commenced his experiment with attached leaves of *Helianthus annuus** at 5 A.M. on August 13, 1883, by cutting seven half-leaves from one large plant. The other halves he took at 3 P.M., after 10 hours of clear sunny weather with blue sky. The day was warm; the highest temperature he records was 25° C. at 3 P.M.

It is quite certain that a shrinkage of 2 or 3 per cent. during this experiment would have been overlooked, while if Sachs used robust leaves, similar to Leaf 11 described above, he might easily have allowed a change of area of 4 or 5 per cent. to pass unnoticed.

Perhaps, however, it will be better to take a general average from actual observations. The following table contains my measurements made in the summer of 1908 under weather conditions which were in several cases less severe than those holding during Sachs' experiment :—

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* *Loc. cit.*, p. 23.

Table XVII.

	Maximum temperature in degrees C.	Weather.	Average percentage decrease in area of Leaves I—IV between 5 A.M. and 3 P.M.
July 22	25·3	Clear	2·8
„ 24	26·6	Hazy	1·4
„ 26	24·4	Bright: white clouds..................	2·3
„ 27	23·8	Clear, changing to rather cloudy, then hazy	3·0
„ 30	27·6	Clear: some wind	5·6
August 3	22·2	Clear	7·3
	Average of all six days		3·7

Applying such a medium correction to Sachs' result, we obtain the following:—

	Sachs' results.	Results "corrected" for shrinkage.
	grammes.	grammes.
Dry weight of 700 sq. cm., 5 A.M.............	3·054	3·054
3 P.M.............	3·693	3·556
Gain 10 hours	0·639	0·502
Gain per square metre per hour	0·914	0·717

If 0·7 gramme is really the true result, Sachs' value was too high by 0·2 gramme, *i.e.* by about 30 per cent.

Brown and Morris, in their repetition of this experiment under similar weather conditions, terminated it two hours later than Sachs.* It is possible, therefore, that their smaller result is to be accounted for by the fact that their experimental half-leaves had had two hours longer in which to recover from shrinkage.†

Sachs' experiment with *Rheum*‡ is also liable to a great error. In this case he commenced at 6 A.M. and ended the experiment at 11 A.M. after five hours of prevailing sunshine. He obtained for the gain of dry weight per square metre per hour the value 0·652 gramme.

In one of my experiments, when the sunshine was only intermittent, four marks were made on a large Rhubarb leaf, roughly in the positions which would be occupied by the four corners of a 100 sq. cm. templet laid upon the leaf. A decrease in the enclosed area of 2·5 per cent. was observed between 8 A.M. and 1.30 P.M. Such a change during Sachs' experiment would have meant an over-estimate of the gain per square metre per hour of 0·16 gramme, *i.e.* 33 per cent., the "corrected" result being 0·49 gramme.

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* *Loc. cit.*, p. 627.

† It may not be entirely fortuitous that their result, 0·713 gramme per square metre per hour, agrees closely with the value 0·7 obtained above by introducing a correction for shrinkage into Sachs' result.

‡ *Loc. cit.*, p. 24.

The relative magnitude of the error introduced by a given change of area is greater the smaller the total photosynthetic increase, the shorter the time of experiment, and the greater the average dry weight of the particular leaves used. On each of these considerations Sachs' experiment with attached leaves of *Cucurbita pepo** is liable to have been vitiated by shrinkage errors to a far greater extent than those with *Helianthus* and *Rheum*.

The average dry weight per unit of area of the *Cucurbita* leaves was greater than that of his *Helianthus* leaves (56 grammes as compared with 44 grammes per square metre), and as the experiment lasted only three hours, the total increase to be expected was comparatively small, and any error was distributed over 3 hours instead of 10 as in the *Helianthus* experiment. In addition to this, the second halves were taken at noon, just the time when, as a rule, the shrinkage is most pronounced.

Sachs commenced his experiment at 9 A.M. on August 21, the temperature rising from 18° C. at that hour to 24° C. at noon, and sunshine continuing throughout. He found an increase of 0·68 gramme per square metre per hour.

Under similar (not extreme) conditions I have observed a shrinkage in area of 3·6 per cent. in a leaf of *Cucurbita*. A change of area by this amount would mean a corresponding apparent increase of 0·7 gramme per square metre per hour, so that *the whole of the observed difference might have been due to shrinkage.*

From these considerations it is clear that the results of all Sachs' experiments with leaves attached to the plant are open to doubt; they may all be too high to a greater or less degree.

Sachs' Experiment with Detached Leaves of Helianthus annuus.

The high result which Sachs obtained for *detached* leaves† suggests the possibility that here, too, shrinkage may have introduced a considerable error, especially as Brown and Morris's results for detached leaves‡ were much smaller. When, however, the details of the experiment are examined, this seems less probable.

Sachs cut eight leaves of *Helianthus annuus* at 5 A.M. and set them in water in the laboratory in dull light till 8 A.M. He exposed the experimental halves in the garden with their stalks in water from 8 A.M. till 2.45 P.M. Finding then that they were flaccid, he stopped the experiment and immersed them in water for half an hour to make them turgescent.

The following experiment shows that this treatment probably eliminated shrinkage:—

Three leaves were detached and left for an hour in a dull light with their stalks in water. After measurement of the distances between marks on the leaves, in the way already described,* the leaves were made flaccid by exposure to sun and wind, and the measurements repeated. At this stage Leaves 1 and 2 were very flaccid, Leaf 3 much less flaccid but drooping. They were then immersed in water and measured after a quarter of an hour, and again at the end of an hour's immersion. The measurements are given in the following table :—

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* *Loc. cit.*, p. 23.

† *Loc. cit.*, p. 25.

‡ *Loc. cit.*, p. 628.

Table XVIII.

Column (1)	Leaf 1, transverse.
„ (2)	„ 1, along midrib.
„ (3)	„ 2, transverse, left half-leaf.
„ (4)	„ 2, „ right „
„ (5)	„ 2, along midrib.
Columns (6), (7), (8)......	„ 3, as for Leaf 2.

Numbers in centimetres.

	Leaf 1.		Leaf 2.			Leaf 3.		
	(1.)	(2.)	(3.)	(4.)	(5.)	(6.)	(7.)	(8.)
(*a*) Initial dimensions	14·51	16·33	9·40	12·50	9·30	8·87	8·76	13·97
(*b*) When flaccid	13·64	16·02	8·60	11·69	9·19	8·70	8·57	13·91
After immersion in water for—								
(*c*) $\frac{1}{4}$ hour	13·73	16·15	8·82	11·79	9·19	**8·92**	**8·82**	**14·01**
(*d*) 1 „	**14·58**	**16·38**	**9·47**	**12·48**	**9·28**	8·92	8·87	14·06

It will be observed that even Leaves 1 and 2, which had been so flaccid that Sachs would probably have rejected them, more than recovered in an hour's immersion. Leaf 3, which had been but moderately flaccid, recovered in a quarter of an hour. It is highly probable, therefore, that Sachs' experimental half-leaves were completely recovered after the half hour's immersion which he gave them. It follows that no deduction can be made from his result on the ground of a shrinkage error. After all possible deductions have been made it still remains substantially the same, still remarkably high.

It may be mentioned here that experiments have been carried out which support Sachs' result.† The full description of these experiments, and a discussion of their relation to Brown and Escombe's experiments, will be postponed till more evidence has been obtained.

Shrinkage Phenomena in General.

It remains to consider the phenomena of shrinkage as they appear in other plants than *Helianthus annuus,* and to discuss in a more general way their bearing on the dry-weight method.

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* See p. 23.

† See Thoday, D., "On Increase in Dry Weight as a Measure of Assimilation," 'Report of the British Association, Dublin,' 1908, p. 905.

In the first place, to indicate how universal are the phenomena in greater or less degree, the following selection is given from measurements of a number of leaves of various types.

Table XIX.—Percentage Decrease in Area (estimated) of various Leaves during Insolation.

August 9, 1908, 6 A.M. to 12 noon. Bright warm day, occasional clouds.

Cercis, four leaves	1·6
	1·6
	2·4
	2·0
Nicotiana, two leaves	1·8
	4·4
Cucurbita	5·6*
Vitis	0·6
Cherry Laurel, three leaves	1·2
	1·2
	0·9

August 10, 1908, 8 A.M. to 1.30 P.M. Cloudy, occasional spells of sun.

Cucurbita, three leaves	1·4 1·0
	2·6 2·6
	2·5*
Rheum, two leaves	2·5
	1·6
Sugar Beet, three leaves	0·6
	1·7
	3·1
Saxifraga, sp. with large thick fleshy leaves	0·8

Shrinkage is shown by all the leaves examined, though in varying degree. Thus, while *Cucurbita* decreases in area by 5·6 per cent, *Vitis* decreases by but 0·6 per cent. Leaves of the same plant may also show considerable differences from one another (*Nicotiana*, Sugar Beet), as was found to be the case with *Helianthus annuus*.†

Xerophytic leaves show relatively little shrinkage (Cherry Laurel, *Vitis*, *Saxifraga*), partly owing to slower evaporation, partly to the mechanical resistance of cuticle and internal strengthening tissue to contraction. Where a leaf is also thick and fleshy (*Saxifraga*) or possesses tissue for water storage, a considerable diminution in thickness might take place before much change of area would be effected.

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* These two results were given by the same leaf, and afford a relative estimate of the conditions which held on the two days.

† See p. 22, etc.

A uniform close-meshed network of veins, such as occurs in *Cercis* (and in Lime), also resists shrinkage. In leaves which shrink most, on the other hand, the ultimate ramifications of the veins are more slender and delicate (*Nicotiana*, *Cucurbita*).

The conditions on August 9 were fairly severe, but *Vitis*, *Cercis*, and the Cherry Laurel gave to the eye no sign whatever of decreased turgidity. Yet the *Cercis* leaves had shrunk on the average nearly 2 per cent., and Cherry Laurel over 1 per cent. *Vitis* is the most resistant of all the leaves observed.

The conclusion from all the results is that shrinkage is a general phenomenon, and can occur to the extent of 2 or 3 per cent., or sometimes more, without very obvious signs of flaccidity accompanying it. It is, therefore, highly desirable from the point of view of the half-leaf dry weight method that shrinkage should be eliminated or corrected for.

Treatment of Detached Leaves.

Greater care to ensure turgidity during experiment might be useful in many cases; but this is difficult in the sun, as all observers have noted. The difficulty is especially great with detached leaves, and most of those who have employed them have worked either entirely in the shade or with translucent screens.

To modify the illumination is, however, not always admissible; but another factor, the water supply, is amenable to control. In cutting leaves from the plant, air is apt to enter and block the cut vessels, and so hinder the passage of water to the lamina.

Sachs' method of collecting his material in the early morning when there was little transpiration is the most efficient way of avoiding this.

Brown and Escombe, gathering their leaves later in the day, cut through the petioles under water.* In experimenting with *Helianthus tuberosus*, however, even leaves so treated were found to droop very rapidly in the sun. If, on the other hand, freshly boiled distilled water was used, they remained turgid much longer.

Steaming the cut end of the petiole is sometimes of advantage. Its success may be due either to the driving of air from the vessels or to the killing of the tissues and the surrounding of the vessels on all sides with available water. The treatment is objectionable, however, since there is danger of secondary injurious effects. Moreover, it is not always more successful than careful cutting under water.

It is probable that almost the whole difficulty in the treatment of detached leaves is due to the blocking of vessels by air: for in an experiment with *Helianthus annuus*, for which leaves were cut from the plants at 5.45 A.M., while still wet with dew, the use of freshly boiled distilled water was quite sufficient to ensure, in three leaves out of five, a degree of turgidity even greater than would have existed on the plant itself under similar conditions.

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* *Loc. cit.*, p. 57.

The use of air-free water is undoubtedly an advantage. In the sun the leaf is heated to a high temperature, and, if well aerated water is used, air is set free in the vessels. Air-free water, on the other hand, may even dissolve any air bubbles that already exist in the water channels.

The water supply to attached leaves may be improved by watering the ground thoroughly. Even this might be inadmissible in œcological investigations, and there are obvious objections from the same point of view to shading the leaves from the full intensity of the sun. Even in investigating some questions of pure physiology shading is to be avoided; but for many purposes this plan could with advantage be adopted as an accessory means.

It is also possible, with attached leaves, to choose the time of the experiment in such a way as to ensure approximately the same degree of turgidity at the beginning and end of the experiment. This would often be difficult and uncertain, however, as under different weather conditions the duration of shrinkage and rate of recovery vary enormously.

Elimination of Shrinkage Errors.

We will now turn to questions of more immediate importance, and discuss methods of completely eliminating or correcting for change of area.

A. *Immersion in Water.*

The most obvious way of eliminating shrinkage errors is to bring the experimental half-leaf to its original condition of turgidity before determining the area.

The experiment with leaves of *Helianthus annuus* testing the effect of soaking them in water, and other similar experiments, suggested that care in avoiding flaccidity, combined with immersion of both control and experimental half-leaves for half an hour before cutting or measuring the area, would suffice to eliminate shrinkage errors sufficiently accurately for many purposes.

Other experiments have shown, however, that this is not always the case, and have revealed other difficulties. Even when both half-leaves are soaked, the experimental half-leaf after exposure to light may expand too much relative to the control half-leaf. With a detached leaf of *Dipsacus* sp. a difference of as much as 1·6 per cent. was observed. In an assimilation experiment with detached leaves of *Helianthus annuus* the excess varied between 0·9 and 1·2 per cent.

Such changes as these, of course, introduce a negative error into the uncorrected increase of dry weight, which, though small, is not negligible if the method is to be of much service in solving problems of interest to the pure physiologist.

Another difficulty has presented itself in my experience with *Dipsacus* leaves. The longitudinal dimensions near the midrib may be appreciably different when the midrib is still attached and after it has been removed. The change is fairly small as a rule, but for accurate work it is necessary to take care that the conditions under which the two half-leaves are measured are similar in respect of the tissue tensions existing, as well as of the state of turgidity. When the templet method is used it is sufficient, if the midrib be stout, to cut pieces from the control half-leaf before removing it, for the resistance of the midrib itself will prevent deformation of the experimental half-leaf owing to the relaxing of the tension on one side. Otherwise, the control half may be left attached ; but this is not always convenient, and is out of the question if it is desired to measure the whole of each half-leaf, by the planimeter method. However, under comparable conditions of turgidity the tissue tensions would probably be similar on the two sides, and if the midrib is removed from the experimental half-leaf before measuring it the error is likely to be reduced to a minimum. This was found to be the case in experiments with *Dipsacus ;* the change in the longitudinal measurement of the control half-leaf following its separation from the rest of the leaf was approximately the same as the corresponding change in the experimental half-leaf consequent on the removal of the midrib.

To sum up, if care is taken to prevent the experimental half-leaf from becoming very flaccid, soaking both half-leaves in water eliminates large shrinkage errors; but a more certain and accurate method is desirable.

B. *Correction for Shrinkage.*

An alternative plan, which avoids the uncertainty that attaches to soaking, is to make a correction for shrinkage.

(1) The method of estimating this correction, from changes in linear dimensions, has been described already. Though only approximate it has been used successfully in several experiments. The marks should be placed symmetrically on the two half-leaves, and with reference to the portions to be used.

Measurement between marks on the leaf with a millimetre scale can only be applicable where the leaves used are large enough for the dimensions measured to approach 10 cm.

For small leaves similar methods could be adopted, based upon the use of a scale with a vernier, or of a small portable microscope with a micrometer eye-piece. The use of the microscope, which would involve the attachment of some form of index to the leaf, might prove somewhat clumsy, and would take longer than measurement by eye. Any form of measurement, indeed, must occupy a considerable time, and it would often be quite impossible to measure the large number of leaves necessary to reduce the asymmetry error within reasonable limits. On the other hand, for many purposes a sufficiently accurate estimate of the average change of dimensions could be obtained from measurements of a few representative leaves. I hope to give some further attention later to the question of the most convenient apparatus and technique for this purpose.

(2) The method adopted by Brown and Escombe for measuring the shrinkage of *Catalpa* leaves is also available as a general method for estimating the shrinkage correction. They took prints of half-leaves still attached to the midrib and petiole before and after exposing them to their experimental conditions, and measured these prints with a planimeter. By following this plan a measurement of the degree of alteration in area would be obtained directly, and, subject to the limits of accuracy of measurement by planimeter, would be more satisfactory than the estimation of area changes from changes in the linear dimensions of selected regions of the leaf. It would be less suitable for attached than for detached leaves, since to obtain a print of a leaf while still on the plant would be an awkward process.

C. *The Stamping Method.*

The simplest and most satisfactory method of eliminating all possible errors from change of area is to mark out the area in some way at the very beginning of an experiment. The stamping method described in Section VI has been devised to fulfil this requirement. The principle of the method is to stamp each half of a leaf with a rectangle, by means of a specially constructed rubber stamp, and to cut out with scissors the area so delimited.

This method, considered merely as a means of determining area, is with care probably as accurate as Sachs' templet method, and for many plants must therefore supersede the latter. Further discussion of this new method will be found in Section VI.

In conclusion, it may be said without hesitation that errors from change of area during experiment are by far the most serious and most difficult to deal with of all the errors to which the Sachs dry-weight method in its original form is liable. In consequence of the ignoring of these errors most of the results which have hitherto been obtained with the method require revision and repetition.

The difficulties are not, however, insurmountable. Changes of area can be estimated and corrected for, although the technique involved must be laborious. On the other hand, the stamping method of area determination, by the use of which shrinkage errors are eliminated, is almost as simple in use as Sachs' templet method, and it is hoped will prove widely applicable.

NOTE.—Shrinkage errors affect any method which involves measuring the difference between the amounts of a substance contained at different times or under different conditions in unit area of leaf surface. The error is relatively great only when the fluctuations are of a lower order of magnitude than the average content. Thus, when increase in proteid content is to be measured, shrinkage errors might be almost as serious as they are in measuring increase of dry weight : on the other hand, they could probably be ignored in determining changes in the amounts of starch and other carbohydrates.

A case which is probably an illustration of this difference in relative magnitude of shrinkage errors is to be found in Menze's comparison of increase of dry weight with gain of carbohydrates (*loc. cit.*, p. 37). Although the conditions under which he conducted his experiments were not such as would tend to produce large area changes, his ash determinations favour the assumption that some area changes did occur : if so, an appreciable part of the excess increase of dry weight which is unexplained by the gain of carbohydrates might be only apparent. On the other hand, some of the surplus probably represents proteid formed, and the fluctuations in ash content may have been in part or wholly real (*cf.* Section III, p. 9, footnote *).

A better illustration is afforded by Saposchnikoff's results (*loc. cit.*, see p. 10, *et seq*). He estimated proteids, as well as starch and soluble carbohydrates : nevertheless, the increase of dry weight was still not completely accounted for, and he fell back on cellulose as the possible form of the unexplained excess. It is much more probable that most of the excess represents apparent increase of dry weight due to shrinkage. The high rates of increase indicated by some of his shorter experiments favour this interpretation.

Section VI.—On the Measurement of Area.

The only important methods hitherto used for determining the area of the portion of leaf of which the dry weight is required are Sachs' templet method, and the planimeter method, used by Brown and Escombe, consisting in the measurement of a photographic print by means of a planimeter.

Two new methods have been devised in the course of this research. One is the punch method, a modification of the templet method depending upon the use of a rotating punch; the other is the stamping method, by which shrinkage errors are eliminated.

The Templet Method.

In this method a piece is cut from the leaf of the same area as a rectangular plate of wood, metal, or glass laid upon it. This is by far the simplest and most rapid method.

Sachs gave the error involved in his use of the templet as a few square millimetres in an area of 50 or 100 square centimetres, or only a few ten-thousandth parts of the whole.* This must, however, be taken as a rough estimate.

Müller† states that in cutting an area of 40 sq. cm. with a templet the limit of the error introduced into the dry weight per square metre varies from 0·25 to 0·66 gramme, according to the prominence of the veins. This is of the order of 1·5 per cent. and much too high. Since he does not say how he arrived at the estimate, no weight can be attached to it.

There is no perfectly satisfactory method of measuring the degree of accuracy of the templet method directly. For instance, to cut out a given area from paper with a templet and then measure it accurately would only give a minimum value for the error involved in cutting a similar piece from a leaf. The leaf is held firmly only at the veins, and it is difficult to avoid some small displacement of the extensible tissue which intervenes.

To obtain a *maximum* estimate of the error, pieces were cut in the usual way from leaves, and photographic prints of these pieces taken immediately on "gelatino-chloride" paper. The untoned prints were subsequently measured by means of a scale of centimetres with a vernier reading to 0·1 mm. A number of measurements were made of the longitudinal and transverse linear dimensions, and from these the area of the print was estimated.

Before discussing the results obtained in this way it is necessary to remark that the only point of immediate importance is the degree of concordance between results obtained with similar leaves. The area of the pieces at the time of printing was likely to be different from their area when the templet was laid upon them and the cuts made, owing to freedom from tension and some loss of water by evaporation. It is, therefore, in accordance with expectation that the areas of leaf pieces given below are all less than the area of the templet used in cutting them; and also that this absolute error is greater for *Helianthus annuus*, which has been shown to shrink so rapidly.

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* *Loc. cit.*

† "Die Assimilationsgrösse bei Zucker- und Stärkeblättern," 'Jahrb. f. wiss. Bot.,' vol. 40, 1904, p. 443.

Accordingly the results for each kind of leaf will be considered separately. They are briefly set forth below.

Two templets were employed, measuring respectively 8 cm. by 5 cm., and 4 cm. by 5 cm. When accurately measured, their areas were found to be 40·2 sq. cm. and 20·1 sq. cm.

Bucklandia populnea: leaves smooth and leathery, with prominent principal veins.

(a) *Templet,* 40·2 *sq. cm.*

40·1 sq. cm. } Rectangles cut from the same leaf and printed together in the
40·0 „ } same frame.
[*]39·9 sq. cm. } Rectangles cut from the same leaf and printed together in the
39·8 „ } same frame.
Maximum difference from the average ±0·15 sq. cm., *i.e.* ±0·4 per cent.

(b) *Templet,* 20·1 *sq. cm.*

20·0 sq. cm. } Printed together.
19·9 „ }
20·0 sq. cm. } „
19·9 „ }
Maximum difference from the average ±0·05 sq. cm., *i.e.* ±0·3 per cent.

Omalanthus Leschenaultianus: leaves softer than those of Bucklandia, but smooth and fairly firm, with prominent principal veins.

Templet, 40·2 *sq. cm.*

40·1 sq. cm. } Printed together.
40·1 „ }
Difference, nil.

Ranunculus sp.*:* leaves large, with soft irregular lamina between a prominent network of veins; specially selected as *unsuitable* for use with templets. The pieces were cut and printed separately.

Templet, 40·2 *sq. cm.*

40·2 sq. cm.
40·1 „
40·0 „
Maximum difference from the average ±0·1 sq. cm., *i.e.* ±0·3 per cent.

Templet, 20·1 *sq. cm.*

19·9 sq. cm.
19·9 „
Difference, nil.

Helianthus annuus: leaves large, fairly thin, with minor veins somewhat outstanding, but otherwise lamina fairly flat. Printed in the summer; this fact and the rapid shrinkage of the leaf account for the low average area of the prints.

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[*] This was a younger leaf than the first, and it is quite conceivable that it should be considered separately, on the same grounds as with leaves of different species. If so, the maximum difference is under ±0·2 per cent.

Templet, 20·1 *sq. cm.*

19·7 sq. cm.
19·5 „
19·6 „
19·6 „
} Rectangles printed together in same frame.

Maximum difference from average ±0·1 sq. cm., *i.e.* ±0·5 per cent.

The greatest error revealed here is 0·5 per cent.; and even this is likely to be an excessive estimate of the errors of the templet method itself, since changes of area from decrease of tension and evaporation of water may vary somewhat from piece to piece. The estimate thus includes errors in the method adopted to test the templet method, as well as the real errors of the latter.

It may be concluded, therefore, that the *greatest error* involved in cutting pieces from leaves *with templets as small as* 40 *and* 20 *sq. cm.* is *less than* 0·5 *per cent. of that area;* and in comparing two individual pieces the *maximum* error is less than 1 per cent. Such errors, therefore, only account for a small part of the differences measured in asymmetry tests, which reach maxima of 4 per cent. or more.

The magnitude of the error may be expected to vary with the character of the venation. If outstanding veins are excluded altogether, the error from extensibility is reduced to a minimum. The only other sources of error are the sloping of the cutting instrument, and inaccuracy in following the edge of the templet: such errors are reducible with careful cutting within very small limits. All the errors depend upon the perimeter, and so are less in proportion for larger areas.

Where outstanding veins were few, templets as small as 10 sq. cm. have been used, and the results of the asymmetry tests with *Paulownia imperialis** warrant the inference that the use of larger templets may be a distinct disadvantage from the point of view of asymmetry if veins are included that are at all prominent. These asymmetry determinations include the errors of measurement of area, but the differences are far too great to be accounted for by these errors, and it may safely be concluded that any possible disadvantage from the relatively greater perimeter, when small templets are used, is not to be compared with the advantage of reducing asymmetry by avoiding veins.†

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* Pp. 17 and 18.

† For small leaves, Müller felt that the chance of the cutting error becoming serious if small templets were employed made some other method desirable (*loc. cit.*). He took sunprints of half-leaves, and estimated the area by cutting out the print and comparing its weight with the weight of a known area of the same paper. He found that when equal areas were cut out of different sheets from the same packet, the maximum error was equivalent to 0·52 sq. cm. in an area of 40 sq. cm. This is equivalent to 1·3 per cent. of the total area. It would seem, therefore, even taking his own estimate of the maximum error in the templet method, 1·5 per cent., that the only advantage of his printing method lies in the use of entire half-leaves, instead of smaller areas cut from them; but his estimate is excessive, and the use of small templets is in reality much more accurate.

It would be easy to devise improvements of the templet method which would ensure very accurate cutting. By using thick templets with vertical edges and a cutting instrument with flat blade fixed vertically, capable of being brought down simultaneously on to the whole length of the required cut, all the errors that have been mentioned would be practically abolished. By such an instrument, too, a number of leaves could be manipulated at the same time without any sacrifice of accuracy.

It may be said in conclusion, therefore, that the errors of the templet method, though appreciable when the method is used in its original form, are capable of almost indefinite reduction.

The Rotating Punch.

A modification of the templet method has already been devised for the purpose of cutting small pieces from between veins, while at the same time avoiding the objections to using small templets by ensuring extreme accuracy in cutting. For the details of the construction of the instrument, Dr. F. F. Blackman was responsible. It consists of an adapted watchmaker's drill fitted with a circular cutter revolving on its own axis, and accurately turned *in situ* for this purpose. The cutting tube slides freely in a vertical direction, and the method of procedure is to set it in motion by means of a water turbine and then bring it down on the leaf, so cutting out a disc equal in area to that enclosed by the cutting edge. This can be repeated rapidly, a number of discs accumulating in the interior of the tube. A lateral slit facilitates their subsequent removal.

The Planimeter Method.

The planimeter method was that adopted by Brown and Escombe as a general method.

They estimate the error in their measurement of leaf-prints by a planimeter as "well under 0·1 per cent."[*] This is apparently to be taken as holding for areas as small as 40 sq. cm.,[†] although usually the areas measured were of the order of 100 sq. cm. The absolute error corresponding to their estimate must therefore be well under 0·05 sq. cm.

Where this method was adopted in the present investigation, the instrument used read by a vernier to 0·1 sq. cm. Under favourable circumstances, the maximum error found, due to inaccuracy in following the bounding line, was ± 0·1 sq. cm. in measuring an area of 27·5 sq. cm.[‡] I am therefore inclined to regard Brown and Escombe's estimate as too low.

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[*] *Loc. cit.*, p. 59.

[†] *Cf. loc. cit.*, p. 60, Table IX.

[‡] Besides this error others were found: on testing the instrument the graduation proved inaccurate, and in addition different results were obtained according to the average angle between the arms. It is thus very necessary to test a planimeter carefully before

There can be no doubt, however, that with a carefully tested and thoroughly trustworthy planimeter, reading to the nearest 0·1 sq. cm., this method would be convenient as a general method, and might be used for pieces cut from between the prominent veins of fairly large leaves without the error becoming prohibitive. In this way it would have the advantage over the templet method, that the form of the piece cut out would not be restricted, and a more complete use of the lamina would be possible while still avoiding the veins.

On the other hand, when describing the results which were obtained in testing the accuracy of the templet method, it was pointed out that the area of the prints was less than the original area of the pieces printed, owing to changes of tension and to shrinkage.* The magnitude of such changes will be less for entire half-leaves than for cut pieces; but in any case it is necessary to ensure as far as possible that the conditions of tension in portions to be compared are the same, during printing, and to adopt exactly the same technique, so that the times elapsing between the removal of the half-leaf, or portion, and the finished print are not only as short as possible, but equal. So long as the errors affect the measurement of control and experimental half-leaves in the same degree the absolute error can be ignored, since its effect in the calculation of the result for the unit of area is of the second order of magnitude.

Besides these errors arising from the varying condition of the leaves themselves, there are others which may be called purely mechanical.

The leaves may not be perfectly smooth and level, and the pressure to which they are subjected during printing may vary. The former difficulty is frequently encountered with leaves like *Tropæolum*, which are sometimes waved at the edges, and in such a case it was found necessary to cut triangular pieces from the waved parts, so as to make them lie flat. This is objectionable, both because of the danger of multiplying the circumference error, and from the increased number of planimeter measurements involved in the separate printing of the detached pieces. It is impossible always to exclude leaves which show this waving of the edge, for under the initial conditions of turgidity it may not be shown at all.

If the same printing frame is used for both halves of a leaf, variations in pressure are scarcely possible to an appreciable extent.

The effect of varying pressure is more likely to be of account in the templet method: but on general grounds, and from a consideration of the results of the test described above, it can be assumed that when a leaf is flat the alteration of area caused by pressure of the templet upon the leaf is very small, even if it is at all appreciable; and if the leaf is not flat the error is insignificant in comparison with that resulting from asymmetry. A leaf of *Catalpa* was tested, in which one side was bulged between the veins, while the other side was flat. Pieces cut by the same templet from each side differed in dry weight to the very exceptional extent of 8 per cent. in favour of the bulged side, whereas the average degree of asymmetry for *Catalpa* is ± 3·5 per cent. Such errors as these can be

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using it for accurate work. To distinguish between errors due to lack of skill in following the perimeter and those due to inaccurate graduation, it is sufficient to take a number of successive readings in a clockwise direction, and then to traverse the same part of the scale again in the counter-clockwise direction. By repeating this several times, a number of readings are obtained in each of several successive parts of the scale.

The planimeter was only used in preparing the material for the earliest analyses (Table I, p. 6), and since eight readings were averaged, the resulting error was insignificant.

* Pp. 39 and 40.

avoided by careful selection of the leaves to be used for experiment, and are, therefore, not inherent in the method.

It may be remarked that by cutting small areas, as with the rotating punch described above, the error from slight bulging would be considerably less than when a large templet is used.

The Stamping Method.

The new method, which has been devised to eliminate shrinkage errors, depends upon marking out a known area on each half-leaf before experiment.

Preliminary tests have been carried out with an inked rubber stamp, making an impression of a rectangle measuring 5 cm. by 2 cm. The areas so marked out were cut from the leaf with scissors. To test the accuracy with which this can be done, a number of impressions were made on a piece of paper, cut out, and measured by means of a scale with a vernier reading to 0·1 mm.

In cutting, the outside of the line was followed, and hence a positive difference from the actual area of the rectangle marked out was always found, but the degree of uniformity which can be assured by a constant procedure is the question of most interest and importance.

The results were as follows:—

Area marked out.	Areas found after cutting out.
10·94 sq. cm.	11·09 sq. cm.
	11·07 ,,
	11·09 ,,
	11·11 ,,
	11·04 ,,
	11·10 ,,
Average	11·08 ,,

The difference between the extreme values is here 0·6 per cent. of the total area. Considering the smallness of the area, this compares very favourably with the templet errors as estimated above.*

Similar tests have not been applied directly, using leaves, as in doing so the same difficulties present themselves as were met with in testing the templet method. Experience with Cherry Laurel, however, has shown that to follow a line with scissors is easier with a soft leaf than with tougher paper; and it is believed that, especially when larger areas are used, this method will be quite as accurate as, if not more accurate than, the templet method. It will be employed in some projected experiments with the dry-weight method by which the solution of some of the more important problems of assimilation in the open air will be attempted.

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* Pp. 39—41.

Section VII.—On the Killing and Drying of Leaf Material.

In view of Brown and Escombe's suggestion that the colloids of the leaf may undergo an alteration in their power to retain water, it was essential that special care should be taken to ensure the complete drying of material to be analysed by combustion.

Brown and Escombe dried their *Catalpa* leaves at 100° C. in a current of dry air or dry hydrogen. As dry leaf material is extraordinarily hygroscopic, I have used a modification of this method in which all contact with the outer air is avoided until the material has been weighed.

The pieces of leaf were first killed by steam as Sachs recommended,* then dried roughly in a water oven; and, finally, dried at 100° C. in a current of dry air in a special glass apparatus enclosed in a steam bath.†

The requirements for subsequent analysis made it desirable to use comparatively small quantities of material; therefore, to avoid the relatively great loss which powdering would entail and the trouble of estimating it, the material was so treated that it would easily fit the porcelain boat while remaining intact.

This was done in two ways. In using the templet method the pieces of leaf, after being cut out, were rolled up into cylindrical form, retained in this form by a spiral of platinum wire, and killed by suspension in steam. In the process of killing, the roll shrank in diameter and became flaccid, and could be removed with ease, without fear of its becoming unrolled. It was then dried in a drying oven, on a curved metal support. Care was taken to put the free edge below, and this precaution, aided by the curvature of the support, prevented the roll from curling out of shape in drying.

The other method of preparation was connected with the use of the rotating punch. The circular cutting edge had a diameter of about 1 cm., and the discs so cut from one half-leaf were threaded on a thin weighed glass needle. After killing, by suspension in steam, a number of such needles of material were put into the ordinary drying oven in special supports made by sticking a number of short narrow pieces of glass tube radially into a bung, the needles fitting into the radiating tubes.

The apparatus in which the final drying took place (see fig. 8) was of such a shape and size as would conveniently accommodate the porcelain boat in which the material was to be burned, so that it could be dried finally in the boat immediately before analysis. The air was heated, before entering the wide tube (B) in which the leaf material was placed, by passing through a narrow tube (A) sealed to the wide tube and spirally coiled round it. Both tubes passed through the same cork (C), which supported them in a specially constructed steam bath (G). The whole formed a convenient and compact piece of apparatus. To avoid contact with the outer air between drying and weighing, the material was weighed in a tube which fitted into the open end of the wide drying tube (D); the joint was made fast by a short length of wide rubber tubing (E), and air, dried by passing through tubes containing calcium chloride and sulphuric acid, was drawn through the whole by means of a water pump with a water resistance to keep the suction force steady. After drying, the material was tilted back into the weighing tube, which was then removed, closed at both ends, and left to cool in a desiccator. When cool, the tube was opened for a moment to equalise the pressure within and without, and weighed closed, and again weighed after removing the material.

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* There is a theoretical objection to putting pieces of leaf straight into the oven in which the preliminary drying is to take place, for the rate at which the material is heated up is relatively slow, and respiration, which increases greatly with the temperature, must entail some loss. The error is probably small, especially when the portions to be compared receive identical treatment. On the other hand, the rapidity with which steam kills the material, owing to its high latent heat, ensures the reduction of all such errors to a minimum. This method has been adopted for all except a few early experiments, and identical treatment given in every respect to portions which were to be compared.

† Heating to 108° C. in a toluol bath drove off no more water.

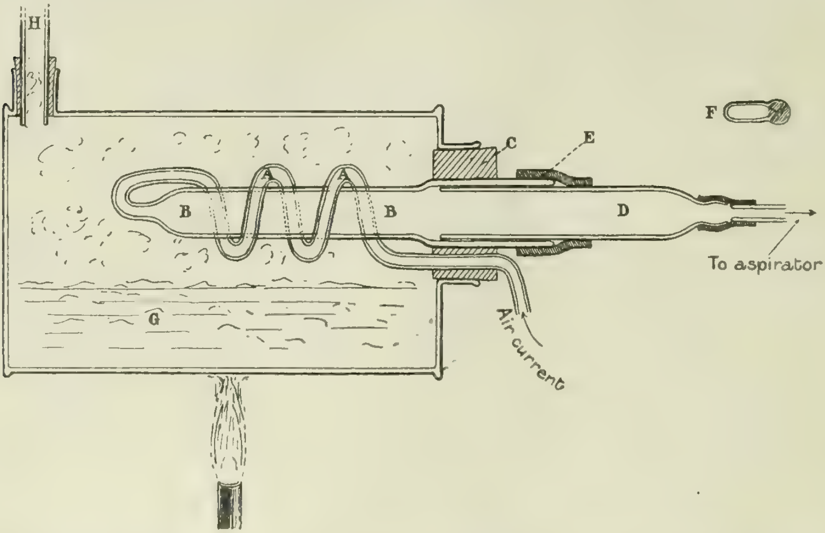

Fig. 8.—A, spiral tube in which air is heated in passing to B, the wide tube in which leaf material is placed to be dried. A and B pass through same cork, C. D, weighing tube, fitting into wider open end of B. E, short length of wide rubber tubing, making D fast to B. F, glass stopper for small end of D; the other end is closed by a rubber stopper. G, cylindrical steam bath. H, glass tube functioning as condenser.

By this method 0·1 to 0·3 gramme of leaf material was often dried and weighed repeatedly without the successive weights obtained varying by more than one-fifth of a milligramme.

After the material had been dried roughly in an ordinary water oven, it was found sufficient for most purposes, for instance in investigating the asymmetry of leaves, to pass a moderate current of air for 15 to 30 minutes before weighing; but the material analysed by combustion was always very carefully dried, usually for an hour at first, and then two or three times for periods of half an hour to ensure absolute constancy of weight.

A really satisfactory method of drying having been devised, attention was turned to the methods which were used by Sachs and others.

Some comparisons have been made of the results obtained by drying in an ordinary water oven and weighing in glass weighing bottles, with the results found after drying at 100° C. in a current of dry air. In making them it was found to be impossible to dry to a constant weight in the oven, and the portions of material from two halves of a leaf were, instead, dried together for the same length of time and treated as nearly as possible in the same way in every respect.

The results of four such experiments are given in the following table. The materials used in two of these were obtained from assimilation experiments with *Tropæolum majus*, in the other two from translocation experiments with *Helianthus tuberosus*.

The areas in the experiments with *Tropæolum* were measured by the planimeter method. Any error entering into the determination of the area does not, of course, affect the comparison.

Table XX.—Comparison between results obtained with a drying oven and with a current of dry air at 100° C.

Weights in grammes per square decimetre.

	Area, sq. cm.	Oven.		Air current.	
		Dry weight.	Difference.	Dry weight.	Difference.
Tropæolum majus I (*a*)	46·2	0·2063	+0·0116	0·1978	+0·0110
(*b*)	41·8	0·2179		0·2088	
„ „ II (*a*)	40·9	0·2303	+0·0019	0·2220	+0·0021
(*b*)	44·9	0·2322		0·2241	
Helianthus tuberosus I (*a*) ...	60	0·4541	−0·0430	0·4448	−0·0440
(*b*) ...	60	0·4111		0·4008	
„ „ II (*a*) ...	50	0·4400	−0·0334	0·4300	−0·0340
(*b*) ...	50	0·4066		0·3960	

These results show that although after drying in the oven a further 4 per cent. of water may be driven off when the material is dried in the current of dry air at 100° C., yet the difference in the values for the gain of dry weight is small. The greatest difference is 0·001 gramme per square decimetre, which is less than 0·2 per cent. of the total dry weight.

Considering the magnitude of the errors from other sources, this is a fair degree of approximation, so that the water-oven method of drying is permissible for rough experiments if care is taken to treat in exactly the same way the two portions of material to be compared, and, preferably, to give them their final drying together. It is not to be recommended if a current of dry air or other gas can be used.

The apparatus described here is not adapted either in form or size for very general use, although it has served the present purposes admirably. When a comparatively large quantity of material from a number of leaves is to be dealt with, a modification of Broocks' two-necked bottle ("Liebig'sche Ente")* would probably be more suitable, the current of dry air passing in at one opening, and out at the other, over the leaf material cut up into pieces of moderate size.

Two other points in Broocks' method are worth noting. He used a current of dry coal gas, and a temperature of 115° obtained with a paraffin bath. The high temperature, although unnecessary, probably increases the rapidity of drying, and the gas current could be used with great economy by passing it, carefully dried, over the material on its way to the burner.

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* *Loc. cit.*, p. 16.

Section VIII.—GENERAL CONSIDERATIONS ON THE DEGREE OF ACCURACY AND THE UTILITY OF THE METHOD.

An attempt will be made in this section to estimate the degree of accuracy which can be obtained with the dry-weight method, and its probable utility in the future.

Since our knowledge of the composition of the true photosynthetic increase of dry weight is at present scanty, attention will be confined to the question *how accurately the gain of dry weight itself can be measured by the half-leaf method.* For this purpose it is only necessary to consider the errors due to asymmetry and change of area, as errors of technique are reducible within very narrow limits and are always included in measurements of asymmetry.

It will be interesting to begin by supposing that in an experiment lasting 5 hours it is desired to measure the increase of dry weight per hour per square decimetre correct to the nearest milligramme, a degree of accuracy which would be sufficient to allow a great deal of work to be done by the method;[*] and to consider the means necessary to secure this degree of accuracy.

For results correct to the nearest milligramme the total error in the increase per hour must not exceed ±0·5 milligramme. If the experiment last 5 hours, the error in the total increase of dry weight during that time must be within ±2·5 milligrammes per square decimetre.

The percentage error to which this limit corresponds will vary with the average dry weight per square decimetre of the leaf. This is widely different for different plants, as the following figures show:

Average Dry Weight of 1 sq. decimetre of various Leaves.

Alliaria officinalis …………	0·18 gramme.	[†]
Helianthus annuus……………	0·33 ,,	[†]
Rumex sp. ……………………	0·41 ,,	[†]
Catalpa bignonioides…………	0·45 ,,	
Paulownia imperialis ………	0·6 ,,	
Cherry Laurel ………………	1·0 ,,	

It may be instructive to calculate the desired limits of the probable percentage errors for *Helianthus* and the Cherry Laurel, as examples of two very different types, the latter somewhat extreme. With the former an error of ±2·5 milligrammes is ±0·8 per cent. of the dry weight of a square decimetre. With the latter it is ±0·3 per cent.

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[*] Brown and Escombe (*loc. cit.*, p. 61) infer from their own results with *Catalpa bignonioides* that a total error from asymmetry and shrinkage of ±2 per cent. of the total dry weight is easily possible, which, in a 5-hour experiment, would mean an error in the increase per square decimetre per hour of ±2 milligrammes; this is equal to the average rate of assimilation found by them for leaves of this plant by measuring the actual intake of carbon dioxide from ordinary air.

As a representative illustration this is unsatisfactory. *Catalpa* leaves are less symmetrical than the average, and there can be no doubt that a rate of assimilation of only 2 milligrammes per hour is greatly exceeded by many leaves in the open air (*cf.* Thoday, D. 'Brit. Assoc. Report, Dublin,' 1908).

[†] Müller, *loc. cit.*, p. 474.

When the stamping method is employed, whereby shrinkage errors are entirely eliminated, the asymmetry error alone remains. In this case, the maximum error from asymmetry must be less than ±0·8 per cent. of the total dry weight for *Helianthus*, less than ±0·3 per cent. for Cherry Laurel, and the *probable* error not greater than about ±0·5 per cent. and ±0·2 per cent. respectively.

The average degree of asymmetry to be expected in individual leaves is about ± 2 per cent. Apart from the possibility of reducing asymmetry errors by avoiding prominent ribs, for which some experimental evidence has been given, the other means of reducing them is to use a large number of leaves. The probable error is thus reduced in the inverse ratio of the square of the number of leaves used. To bring the average error from asymmetry within the required limits, 16 leaves of *Helianthus*, or 100 leaves of Cherry Laurel, would therefore be required.

There are obvious practical objections to using a very large number of leaves, since the time taken in cutting out stamped areas with scissors is a consideration of real importance; as also in cutting out pieces by means of templets, or in taking photographic prints.

Moreover, with large-leaved plants like *Helianthus annuus*, bearing comparatively few leaves of which but a small proportion are perfectly sound and flat, the necessity of using such a large number of leaves would be a great drawback.

One hundred leaves of Cherry Laurel, too, would be quite unmanageable by the stamping method. On the other hand, with an improved templet or the rotating punch, it would be possible to manipulate a number of these fairly firm smooth leaves at the same time. With such leaves, which as a rule also show but a small degree of shrinkage under insolation, these methods are to be recommended in preference to the stamping method, a correction for area changes being estimated from measurement of representative leaves.

The accuracy with which shrinkage can be allowed for by measurement can only be roughly gauged. The error remaining after approximate correction is probably well within ±0·5 per cent. of the total area, and therefore less than one-fifth of that due to asymmetry.

The conclusion seems unavoidable, nevertheless, that the probable error from all sources can in general only with difficulty be reduced, in a five-hour experiment, to so low a figure as ± 0·5 milligramme per square decimetre per hour. To attain to this degree of accuracy without making the method too laborious, *the time of experiment must be increased, or the average degree of asymmetry for individual leaves reduced by avoiding veins.*

Thus if the average degree of asymmetry were for *Helianthus annuus* reduced to 1·5 per cent., that is by $\frac{3}{4}$, and the time of experiment increased to 10 hours, the number of leaves required, for the same degree of accuracy which demanded 16 leaves under the conditions originally assumed, would be reduced to $16 \times (\frac{3}{4} \times \frac{5}{10})^2$; that is, to two or three.

The number of leaves of *Helianthus annuus* used by Sachs for each experiment was seven or eight, and the same number gave Brown and Morris's difference of 1·1 per cent., which for a 10 hours' experiment works out to 0·4 milligramme per hour, and thus within the ± 0·5 milligramme error for which we have been calculating.

It will be convenient in this place to examine Brown and Escombe's experiments, in which they compared the apparent increase of dry weight, given by the simple half-leaf method, with the weight of carbohydrate corresponding to the carbon dioxide actually absorbed.

Considerations will be adduced in favour of the conclusion that the great discrepancies which they found are adequately explained by the asymmetry of leaves of *Catalpa bignonioides*, and their shrinkage under the experimental conditions.

Changes in ash content and inadequacy of the "carbohydrate factor" could account for a small part, but their effect may be ignored for the present purpose.

Before considering the results of the experiments themselves, it is necessary to estimate the probable magnitude of the errors. Unfortunately, this can only be done in a general way, as Brown and Escombe give neither the conditions under which the individual experiments were performed nor the time during which each lasted.

An instance of the possible *error from asymmetry* can be derived from the results of their own asymmetry test.* In each of their dry weight experiments they used four leaves, taking as control half-leaves the left and right halves alternately. Treating in this way the first four examples of *Catalpa bignonioides* from their asymmetry table, a difference is obtained, when the four leaves are taken together, of 2·1 per cent. The average dry weight per square decimetre of their *Catalpa* leaves is about 0·45 gramme, so that this error would have meant an under- or over-estimate of the total increase of dry weight by 9·4 milligrammes per square decimetre.

The possible errors from *shrinkage* must be deduced from the one experiment† in which Brown and Escombe measured the changes of area taking place in the experimental chamber used in their tests. They found the following percentage differences between the areas of the same half-leaves before and after exposure in the chamber:—

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* Quoted on p. 13.

† *Loc. cit.*, p. 60.

	Per cent.
(1)	−3·12
(2)	+0·98
(3)	+0·36
(4)	+0·14
Average ...	±1·1

The conditions under which these figures were obtained may have differed from those under which the actual assimilation experiments were performed. The leaves for the latter were covered, overnight, with tinfoil, in order to render them starch free. On the other hand, no mention is made of the like treatment of the leaves for this experiment on change of area. If this precaution were really omitted, the results may require a negative correction owing to the less turgid condition of the leaves when first enclosed.

Apart from this, the fact that one leaf had shrunk by as much as 3 per cent. shows that an average difference much greater than 1·1 per cent. might have occurred in some of their experiments. It is interesting in this connection that leaves of *Catalpa bignonioides* were found by Halsted* to shrink to an exceptional extent when dried for the herbarium; and I have observed that leaves of this species show very little sign of flaccidity, even after a shrinkage in area of 4 per cent.

Calculating first from their average change of area of 1·1 per cent., we obtain a possible positive error of 4·9 milligrammes per square decimetre in the total gain of dry weight. An average shrinkage of 3 per cent. would mean an error of 13·5 milligrammes. Adding the asymmetry error, assuming it to be positive, the total positive error becomes 14·3 or 22·9 milligrammes, according to the degree of shrinkage assumed.

This has to be divided by the number of hours, which is not stated Considering, nevertheless, that one of their dry weight experiments† lasted only 3¼ hours, we may perhaps calculate the error per hour on the basis of a 3-hour experiment, as well as of the 5-hour experiment for which their own calculation was made.

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* Halsted, "Shrinkage of Leaves in Drying," 'Bull. Torrey Bot. Club.,' 1894, p. 129.

† *Loc. cit.*, p. 56, Table VII.

The respective errors in the gain per hour per square decimetre are as follows:—

Duration of experiment.	Error, assuming shrinkage to the extent of	
	1·1 per cent.	3 per cent.
	milligrammes.	milligrammes.
5 hours	2·9	4·6
3 ,,	4·8	7·6

Brown and Escombe's results, with which these estimates are to be compared, may now be given:—*

	Increase in milligrammes per square decimetre per hour.		
	Found by dry weight method.	Calculated from intake of CO_2.	Difference.
Experiment 1	9·8	1·8	+8·0
,, 2	7·1	1·8	+5·3
,, 3	2·6	2·9	−0·3
,, 4	7·2	2·9	+4·3
,, 5	Loss of weight.	3·0	Greater than −3·0

Since the greatest discrepancy between Brown and Escombe's gasometric and dry weight results is 8 milligrammes per square decimetre per hour, it is probable that *the very large and very variable errors found by them in the dry weight method are all explainable as due to asymmetry and shrinkage.* These will explain negative errors as well as positive: for asymmetry may have a positive or negative effect on the result; and, as their own experiment shows, changes of area may be positive as well as negative, although the fact that they are in general more often negative accounts for the usual positive direction of the resultant error.

NOTE.—One other fact may be adduced in support of the explanation of these large errors by the occurrence of considerable shrinkage. In Table VII† they give three experiments with *Catalpa bignonioides*, lasting respectively 7, 5, and 3¼ hours.

The results per square decimetre per hour were as follows:—

In 7 hours	7·9	milligrammes
5 ,,	13·7	,,
3¼ ,,	16·6	,,

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* *Loc. cit.*, p. 58, Table VIII.

† *Loc. cit.*, p. 56.

They show a greater increase the shorter the time of experiment, which is just the apparent effect that the division of a positive error over a varying number of hours would produce. The point cannot, however, be pressed, and the fact may have some other explanation ; but it is worth while to mention the possibility, especially as the results are so much higher even than those obtained in the experiments already discussed.

Section IX.—CONCLUSIONS.

1. Carbon analyses have shown that, except for minor differences, the same result is obtained for the rate of assimilation by the half-leaf method, whether increase of dry weight or of carbon content is measured. The dry weight method is therefore not vitiated by any large indeterminable errors such as would arise if varying quantities of water were retained by the colloids of the leaf after drying it at 100° C.

The calculation of the equivalent intake of carbon dioxide from the increase of dry weight can, however, only be approximate, as too little is known of the very variable composition of the products of assimilation. As a rule it will probably be advisable to determine the increase in ash content and deduct it from the increase in dry weight.

2. The tendency of the method to give results which are too high is amply explained by *shrinkage* in area of the experimental half-leaf through loss of turgor during insolation. Shrinkage under insolation is a general phenomenon : it is shown in varying degree by all the leaves examined. Leaves of *Helianthus annuus* often diminish in area by more than 5 per cent. between early morning, when the air is moist, and midday, when the hot sun and dry air favour rapid evaporation. Robust leaves of this plant may show to the eye little sign of flaccidity, even though they are 4 or 5 per cent. less in area than when fully turgid; hence errors from shrinkage cannot be avoided by mere inspection.

The leaves of *Helianthus annuus* are extraordinarily sensitive to changes in the intensity of illumination, and react to them with great rapidity. A leaf was observed to increase or decrease in breadth by nearly 2 per cent. within 10 minutes when the sun passed behind a cloud, or appeared again after having been for a time obscured.

Since this source of error has been practically ignored in all the work hitherto done by the dry weight method, few of the results are trustworthy. Thus the rate of assimilation found by Sachs for attached leaves of *Helianthus annuus*, viz., 9 milligrammes per square decimetre, may easily have been 2 milligrammes in excess of the true rate; while in his similar experiment with *Cucurbita pepo* the whole of the apparent gain may have been illusory.

On the other hand, Sachs' result for detached leaves of *Helianthus annuus*, 16 milligrammes per square decimetre, although so high, is not open to the same criticism, for he soaked the experimental half-leaves in water at the end of the experiment to make them turgid. Soaking in water does in most cases roughly counteract shrinkage errors, but more accurate means must be adopted for general use.

As an alternative, changes of area may be measured by the planimeter method, or estimated from measurements of the changes in longitudinal dimensions, and a corresponding correction then applied to the increase of dry weight. Errors from shrinkage can also be entirely eliminated by the simpler alternative of marking out a given area on both control and experimental half-leaves at the beginning of the experiment. The *stamping method* has been devised for this purpose: in this method an ink impression of a rectangle is made on each half-leaf with a specially constructed rubber stamp.

3. The other important error to which the dry weight method is liable arises from *asymmetry*, from the fact that equal areas, taken at the same time from the two halves of a leaf, have not accurately the same dry weight. This source of error is inherent in the method and cannot be eliminated: it determines the ultimate limit of the accuracy obtainable.

The error can be diminished: (1) by avoiding outstanding veins, and so reducing the degree of asymmetry shown by individual leaves, and (2) by using a number of leaves for each experiment.

In testing leaves of *Paulownia imperialis*, using only parts without prominent veins, differences of the order of 1½ per cent. were found, whereas portions with the minor veins slightly projecting gave differences *four times as great*.

The reduction of the asymmetry error by the use of a large number of leaves is limited in practice. An improved templet, or the rotating punch, would make possible the simultaneous manipulation of a number of leaves of smooth, firm texture; but the result would have to be corrected for shrinkage, and the estimation of this correction would prove laborious unless it could be made from measurements of a few representative leaves. To deal singly with a large number of leaves would take too long, whatever the method employed.

4. Included in measurements of asymmetry are the experimental errors involved in *measuring the area* and finding the *dry weight* of individual portions of leaf material. Although these are small compared with the error from actual asymmetry, it is nevertheless important to reduce them as much as possible, as they are all cumulative in their effect upon the result.

4A. The important methods of area determination are four in number: Sachs' templet method; Brown and Escombe's planimeter method; and the rotating punch and stamping methods devised in the course of this research.

A test applied to Sachs' *templet method* showed an error which was considerably less than 0·5 per cent. of areas as small as 20 sq. cm.: the other methods are at least as accurate as this.

All the errors of the templet method can be reduced to a minimum by modifying the form of the templet and of the cutting instrument.

By means of the *rotating punch* small discs may be cut from between the veins. Since, however, this method is not a very rapid one it should not be used for leaves like those of *Helianthus annuus,* which are liable to rapid shrinkage.

The *planimeter method* can be used for entire half-leaves, or for pieces cut from between the veins; great care is necessary, however, especially in the latter case, to avoid shrinkage while taking the photographic print.

The stamping method completely eliminates all shrinkage errors; by it the dry weight method is made as simple as possible, but its use is restricted to fairly smooth leaves. With both the templet and stamping methods areas as small as 10 sq. cm. can be safely used; but the disc and planimeter methods are more suitable for very small leaves.

4B. Since dry leaf material is often extremely hygroscopic, it should be dried in a current of dry air and weighed with great care to exclude moisture.

5. To sum up, the main errors involved in the dry-weight method are due to (1) the shrinking of leaves in area during experiment, and (2) their lack of symmetry in respect of dry weight per unit area. Of these errors, those from shrinkage can be eliminated; those from asymmetry, on the other hand, must always be reckoned with. *Asymmetry tests must therefore always form an integral part of dry weight experiments;* especially as any appreciable reduction in *experimental* errors will also appear in these tests.

The relative magnitude of the total error may be reduced by increasing the time of experiment and so distributing it over a larger number of hours.

The real increase of dry weight in a period of 5 *hours or more can be determined with most leaves correct to the nearest milligramme per square decimetre per hour by using appropriate methods.** With shorter experiments this degree of accuracy cannot be reached except with very thin leaves.

Thus, the dry weight method is capable of yielding useful results, for rates of increase greater than 2 milligrammes per square decimetre per hour. It is not to be compared for accuracy with gasometric methods, and can never be expected to afford a means of studying the more refined questions of pure physiology; but the accuracy obtainable will allow of the approximate solution of a number of interesting problems, and will be of service in ecological comparative studies of assimilatory activity.

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* This applies strictly only to broad leaves. For narrow linear leaves special methods would have to be devised, and for many very small leaves the half-leaf method is obviously unsuitable.

Sleeping Sickness in Uganda.—Duration of the Infectivity of the Glossina palpalis *after the Removal of the Lake-shore Population.*

By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908—09.)

(Received November 16,—Read November 25, 1909.)

During the last two years the policy of clearing the shores and islands of Lake Victoria of their inhabitants has been carried out by the Uganda Administration, with a view to the stamping out of Sleeping Sickness.

It will be remembered that the area of distribution of Sleeping Sickness and of the *Glossina palpalis* in Uganda is the same, and is limited to a narrow belt along the Lake-shore and islands. For the past two years no native has been allowed to live or work within two miles of the Lake-shore, except at a few cleared landing-places; and within the last few months all the islands have been emptied.

Until recently it was believed that the fly only retained its infectivity for 48 hours, and that it would, theoretically, be possible with safety to clear an island of its infected population one day and restock it with healthy natives a few days later. Recent work, however, has shown this to be wrong, since it has been found by experiment that the fly can retain its infectivity up to 80 days. It is probable that after a fly has become infected it will harbour the trypanosomes for the rest of its life; but what the duration of this is, under natural conditions, is unknown.

From an administrative point of view, therefore, it is most important to find out how long the flies on the Lake-shore remain infective after the native population has been removed. Until this is known it will not be safe to allow the Lake-shore and islands to be re-inhabited.

As soon as the Sleeping Sickness Commission of the Royal Society reached Uganda experiments were begun to test this point. At first the flies were collected at Kibanga, a cleared landing-place in Buka Bay, six miles from the laboratory. This landing-place was used as a market, where the inhabitants of the Island of Buvuma came once a week to trade with the natives on the mainland. In November, 1908, Kibanga had become somewhat overgrown, and tsetse flies were present in some numbers. As the Buvuma islanders were highly infected with Sleeping Sickness, this constituted a danger to the healthy natives of the mainland, who had come to the market from outside the Sleeping Sickness area. Steps were at once taken to have the landing thoroughly cleared of undergrowth, with the result that in a short time the flies disappeared.

The following experiment shows the result:—

Experiment 52.—Monkey.

To ascertain if *Glossina palpalis* caught at Kibanga market-place are capable of giving rise to Sleeping Sickness in a healthy monkey.

Date.	No. of flies.		Trypanosomes.	Malaria.	Date.	No. of flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1908.					1908.				
Nov. 3	—	—	—	+	Dec. 6	—	—	—	+
,, 6	—	—	—	+	,, 7	—	—	—	+
,, 14	15	12	—	+	,, 15	—	—	—	+
,, 16	17	17	—	+	,, 17	1	1		
,, 17	7	7	—	+	,, 18	—	—	—	+
,, 18	4	1	—	+	,, 23	—	—	—	+
,, 19	7	4	—	+	,, 26	—	—	—	+
,, 20	—	—	—	+	,, 30	—	—	—	+
,, 22	50	34	—	+					
,, 23	—	—	—	+	1909.				
,, 24	—	—	—	+	Jan. 4	—	—	—	+
,, 25	—	—	—	+	,, 9	—	—	—	+
,, 27	—	—	—	+	,, 18	—	—	—	+
,, 29	—	—	—	+	,, 20	—	—	—	+
,, 30	—	—	—	+	,, 26	—	—	—	+
Dec. 2	10	7	—	+	,, 28	—	—	—	+
,, 3	12	5	—	+	Feb. 6	—	—	—	+
,, 4	5	3	—	+	Mar. 1	—	—	—	+

Remarks.—The result of this experiment is negative. The number of flies caught is small, and they soon disappeared as the clearing of the place proceeded.

The other experiments were all made with freshly-caught flies from uninhabited places on the Lake-shore. The Lake-shore, as stated above, had been cleared of its inhabitants in December, 1907, and had, therefore, been deserted for nearly a year when these experiments began. It was anticipated that the flies would be found non-infective, as, in the absence of Sleeping Sickness cases, it was difficult to understand where they could obtain the necessary trypanosomes, and at this time the long period of infectivity of the fly was unknown. The following experiments give the result:—

Experiment 214.—Monkey.

To ascertain if *Glossina palpalis*, caught on the Lake-shore, where there are no natives, are capable of giving rise to Sleeping Sickness in healthy monkeys.

Date.	No. of flies.		Trypanosomes.	Malaria.	Date.	No. of flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1908.					1909.				
Nov. 23	21	10			Jan. 4	—	—	—	+
,, 24	25	20			,, 9	—	—	—	+
,, 25	50	26	—	+	,, 15	—	—	—	—
,, 26	30	17	—	+	,, 25	43	24		
,, 27	12	8			,, 26	35	29	—	+
,, 28	96	23	—	+	,, 28	—	—	—	+
,, 30	125	41	—	+	Feb. 2	100	65	—	+
Dec. 1	150	60	—	+	,, 3	105	105		
,, 2	—	—	—	+	,, 4	100	90		
,, 3	—	—	—	+	,, 5	—	—	—	+
,, 4	—	—	—	+	,, 6	100	85		
,, 5	60	23	—	+	,, 8	100	82		
,, 7	47	26	—	+	,, 9	200	165		
,, 12	60	49	—	+	,, 10	200	146	—	+
,, 14	83	37			,, 15	200	135	—	+
,, 15	78	32	—	+	,, 16	200	120		
,, 17	14	6			,, 17	170	126		
,, 18	—	—	—	+	,, 18	200	134		
,, 23	—	—	—	+	,, 19	200	110	—	+
,, 28	80	35	—	+	,, 20	200	124		
,, 30	70	32	—	+	,, 22	130	98		
					,, 23	200	140		
					,, 24	200	142		
					,, 25	200	135		
					,, 26	—	—	—	+
					Mar. 1	—	—	+	+

Remarks.—2500 flies were fed on this monkey for 98 days before a positive result was obtained.

Experiment 571.—Monkey.

Date.	No. of flies.		Trypanosomes.	Malaria.	Date.	No. of flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
Mar. 2	200	152			Mar. 15	200	152		
,, 3	200	156			,, 16	100	78		
,, 4	100	78			,, 17	100	74		
,, 6	150	110			,, 18	100	58	—	+
,, 9	200	120	—	—	,, 20	200	112		
,, 10	200	110			,, 22	—	—	+	+
,, 11	200	124							

Remarks.—Result positive. Infection probably took place on March 15. This means that 1002 flies fed on this monkey before infection took place.

Experiment 612.—Monkey.

Date.	No. of flies.		Trypanosomes.	Malaria.	Date.	No. of flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
Mar. 25	340	185			Mar. 29	100	76		
„ 26	250	124			„ 30	200	115	—	+
„ 27	200	115			April 6	—	—	+	+

Remarks.—Result positive. Infection probably March 30; 615 flies.

Experiment 674.—Monkey.

Date.	No. of flies.		Trypanosomes.	Malaria.	Date.	No. of flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
April 8	250	160			April 23	270	180		
„ 9	500	240			„ 26	200	160	—	—
„ 10	500	220			„ 28	400	240		
„ 12	500	245	—	+	„ 30	400	160		
„ 15	500	340			May 1	500	290		
„ 19	—	—	—	—	„ 3	—	—	—	+
„ 20	250	180			„ 7	—	—	+ +	+
„ 22	300	190	—	—					

Remarks.—Result positive. Infection April 30; 2315 flies.

Experiment 758.—Monkey.

Date.	No. of flies.		Trypanosomes.	Malaria.	Date.	No. of flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
May 8	270	210			May 22	—	—	—	+
„ 11	250	170			„ 28	200	130	—	+
„ 14	200	120			June 2	—	—	—	+
„ 17	—	—	—	+	„ 7	—	—	+	+

Remarks.—Result positive. Infection May 28; 630 flies.

Experiment 976.—Monkey.

Date.	No. of flies.		Trypanosomes.	Malaria.	Date.	No. of flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
June 9	800	260			June 18	200	90	—	+
,, 10	450	180			,, 20	520	230		
,, 17	550	190			,, 21	—	—	+ +	+

Remarks.—Result positive. Infection June 10; 440 flies.

Experiment 1117.—Monkey.

Date.	No. of flies.		Trypanosomes.	Malaria.	Date.	No. of flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
June 24	200	120			June 30	7	4		
,, 25	300	160			July 1	—	—	—	+
,, 26	150	80			,, 3	500	130		
,, 28	380	165			,, 5	500	220	+	+
,, 29	500	210							

Remarks.—Result positive. Infection June 28; 525 flies.

Experiment 1276.—Monkey.

Date.	No. of flies.		Trypanosomes.	Malaria.	Date.	No. of flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
July 9	110	70			July 19	—	—	—	+
,, 12	500	230			,, 20	300	180		
,, 15	—	—	—	+	,, 22	—	—	+	+

Remarks.—Result positive. Infection July 12; 300 flies.

Experiment 1462.—Ox.

Date.	No. of flies.		Trypanosomes.	Malaria.	Date.	No. of flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
Aug. 16	120	75	—		Aug. 20	170	80		
„ 17	410	250			„ 24	350	120	—	
„ 19	320	180	—		„ 26	—	—	+	

Remarks.—Result positive. Infection August 19 ; 505 flies.

Experiment 1465.—Ox.

Date.	No. of flies.		Trypanosomes.	Malaria.	Date.	No. of flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
Aug. 27	150	90			Sep. 7	—	—	—	
„ 28	60	35			„ 9	30	19		
Sep. 4	230	170			„ 10	—	—	+ +	

Remarks.—Result positive. Infection September 4 ; 295 flies.

Experiment 982.—Ox.

Date.	No. of flies.		Trypanosomes.	Malaria.	Date.	No. of flies.		Trypanosomes.	Malaria.
	Put on.	Fed.				Put on.	Fed.		
1909.					1909.				
Sep. 11	45	36			Sep. 20	—	—	—	
„ 12	65	50			„ 21	115	85	—	
„ 14	110	75			„ 22	180	145		
„ 15	125	95			„ 23	410	380		
„ 16	420	160	—		„ 24	300	240	—	
„ 19	55	40			„ 27	370	230	+ +	

Remarks.—Result positive. Infection, September 19 ; 456 flies.

The following table summarises these results:—

Experiment.	Place.	No. of flies fed.	No. of days before infection took place.	Result.	Percentage of infected flies.*
52	Kibanga …………	91	—	—	—
214	Uninhabited Lake-shore	2500	98	+	0·04
571	,, ,,	1002	20	+	0·10
612	,, ,,	615	12	+	0·16
674	,, ,,	2315	29	+	0·04
758	,, ,,	630	30	+	0·16
976	,, ,,	440	12	+	0·23
1117	,, ,,	525	11	+	0·19
1276	,, ,,	300	13	+	0·33
1462	,, ,,	505	10	+	0·19
1465	,, ,,	295	14	+	0·34
982	,, ,,	456	16	+	0·22

* This is calculated on the assumption that there is only one infected fly in each batch of flies used in an experiment.

It must therefore be concluded that the Glossina palpalis *on the uninhabited shores of Victoria Nyanza can retain their infectivity for a period of at least two years after the native population has been removed.* How much longer they will remain infective it is impossible to say, but it is obvious that these experiments should be continued, in order to answer this important question.

With the facts at our disposal it is not possible to account for this continued infectivity. It may be due to the duration of the life of these flies being more than two years—that flies which became infected before the natives left are still alive. Or, it is possible that the flies have lately fed on natives suffering from Sleeping Sickness, who have been passing in canoes from the islands to the mainland, or on natives who still frequent the Lake-shore in spite of the prohibition. Thirdly, it might be explained, if any of our canoe-men or fly-boys had trypanosomes in their blood. Or, lastly, it is possible that the mammals and birds along the Lake-shore have become infected, and so act as a reservoir of the disease.

To these speculations it may be answered that it is not at all likely that these flies have the opportunity of becoming infected from passing canoes, which during the last two years have been few and far between, or to natives still frequenting the Lake-shore. Our canoe-men and fly-boys have been kept under careful supervision during the whole of the time, their blood constantly examined, and once a month blood from each of them injected into a healthy monkey. There remain, then, the two theories—long duration of life of the fly, and a local reservoir. The former cannot at present be answered, and there is no experimental proof of the latter, since the injection of the blood of the Lake-shore birds and mammals into susceptible animals has always, up to the present, given negative results.

Glossina palpalis *as a Carrier of* Trypanosoma vivax *in Uganda.*

By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908–09.)

(Received November 27,—Read December 9, 1909.)

One of the important trypanosome diseases of cattle in Uganda is that caused by *Trypanosoma vivax* (Ziemann). This species of trypanosome appears to be widely distributed in Central Africa. It has been reported from Senegal, the Sudan and Erythrea in the North, to Rhodesia in the South. It is fairly easily recognised on account of its extreme activity during life, its characteristic shape in stained specimens, and the fact that it only affects cattle, goats, and sheep; while monkeys, dogs, rabbits, guinea-pigs, rats, and mice are refractory. Its carriers have usually been reported as tabânus and stomoxys.

This short note is written to place on record that fact, that in Uganda the tsetse flies, *Glossina palpalis*, which are found in large numbers on the Lake-shore, are infected, not only by *Trypanosoma gambiense*, the cause of sleeping sickness, but also by *Trypanosoma vivax*. The first experiment which showed that these tsetse flies are infected with the latter trypanosome was the following:—

Experiment 1318.—Calf.

To ascertain if oxen will become infected by trypanosomes if allowed to feed in the "fly area."

July 12, 1909. A healthy calf was taken down to the Lake-shore at Kibanga and ferried across the bay to Nsonga, where tsetse flies are numerous. The flies were observed to feed on it in numbers. It was then brought back to Kibanga. In future this calf will be taken out every day by the fly-boys to different parts of the Lake-shore, where it will graze while the boys are catching tsetse flies.

August 8. Returned from Lake-shore to Mpumu.

August 11. *Trypanosoma vivax* present in the blood of this calf.

Remarks.—If the incubation period of this disease is assumed to be eight days, then this calf remained 19 days at the Lake-shore before it became infected. The proof that the trypanosome found in this calf's blood was *Trypanosoma vivax* and not *Trypanosoma gambiense* was the shape and appearance of the parasite, the fact that the calf's blood injected under the skin of two monkeys gave negative results, and, lastly, that 50 laboratory-bred flies fed on this calf afterwards infected a goat with *Trypanosoma vivax.*

Experiment 431.—Cow. (Mother of Calf, 1318.)

July 12, 1909. This cow accompanied her calf to Kibanga, and remained with it during the experiment.

August 8. Returned to Mpumu.

August 19. *Trypanosoma vivax* discovered in blood.

The following table shows the dates of examination :—

Date.	Parasites in blood.	
	Piroplasma.	Trypanosoma.
1909.		
January 9	—	—
,, 20	—	—
,, 21	—	—
,, 28	—	—
February 2	—	—
,, 9	—	—
,, 26	—	—
August 9	—	—
,, 11	—	—
,, 19	—	+

Remarks.—It is possible that this cow became infected from her calf, but it is more probable that she became infected in the same way and about the same time as her calf.

The remaining experiments were carried out by bringing freshly-caught *Glossina palpalis* from the Lake-shore to the laboratory at Mpumu and placing them on healthy oxen.

Experiment 1462.—Bull.

To ascertain if freshly-caught *Glossina palpalis*, fed on healthy cattle, will give rise to any trypanosome disease.

Date.	Flies.		Result.	
	Put on.	Fed.	*T. gambiense.*	*T. vivax.*
1909.				
August 16	120	75	—	—
„ 17	410	250		
„ 19	320	180	—	—
„ 20	170	80		
„ 24	350	120	—	—
„ 26			+	—
September 1			—	+
„ 2			—	+
„ 3			—	+
„ 4			—	+
„ 6			+	+
„ 7			+	+
„ 8			+	+
„ 9			—	+

Remarks.—Both *Trypanosoma gambiense* and *Trypanosoma vivax* appeared in the blood of this bull.

Experiment 445.—Bull.

Date.	Flies.		Result. *Trypanosoma vivax.*
	Put on.	Fed.	
1909.			
September 28............	470	220	
„ 29............	160	95	
„ 30............	65	45	—
October 1	300	180	
„ 2	400	280	
„ 4	500	300	—
„ 5	190	85	
„ 7	250	170	—
„ 8	300	165	
„ 11	100	70	—
„ 15	450	360	—
„ 16	470	350	
„ 18	—	—	+
„ 19	—	—	+ +

Remarks.—*Trypanosoma vivax* only appeared in the blood of this bull.

Experiment 1465.—Bull.

Date.	Flies.		Result.	
	Put on.	Fed.	*T. gambiense.*	*T. vivax.*
1909.				
August 27	150	90	—	—
„ 28	60	35	—	—
September 4	230	170	—	—
„ 7	—	—	—	—
„ 9	30	19		
„ 10	—	—	—	+
„ 11	—	—	+	—

Remarks.—Both *Trypanosoma gambiense* and *Trypanosoma vivax* appeared in the blood of this bull.

Experiment 982.—Bull.

Date.	Flies.		Result.	
	Put on.	Fed.	*T. gambiense.*	*T. vivax.*
1909.				
September 11......	45	36		
„ 12......	65	50		
„ 14......	110	75		
„ 15......	125	95		
„ 16......	420	160	—	—
„ 19......	55	40		
„ 20......	—	—	—	—
„ 21......	115	85	—	—
„ 22......	180	145		
„ 23......	410	380		
„ 24......	300	240	—	—
„ 25......	—	—	—	—
„ 27......	370	230	+	—
October 14	—	—	—	+

Remarks.—Both *Trypanosoma gambiense* and *Trypanosoma vivax* appeared in the blood of this bull.

Conclusions.

1. The *Glossina palpalis* on the shores of Victoria Nyanza are infected, not only by *Trypanosoma gambiense*, but also by *Trypanosoma vivax*.

2. What the reservoir of the virus of *Trypanosoma vivax* is, is unknown, but the buffalo, waterbuck, and other antelope which live on the Lake-shore should be examined.

On the Presence of Hæm-agglutinins, Hæm-opsonins, and Hæmolysins in the Blood obtained from Infectious and Non-Infectious Diseases in Man. (Third Report.)

By Leonard S. Dudgeon, F.R.C.P. Lond., and H. A. F. Wilson.

(Communicated by Dr. F. W. Mott, F.R.S. Received September 11,—Read December 9, 1909.)

(From the Pathological Laboratories, St. Thomas's Hospital.)

The further investigations on this subject which form the basis of this paper have been mainly directed towards phagocytosis and the specific hæm-agglutinins and hæmolysins present in the blood of apparently healthy people and those suffering from acute and chronic infections. To these we may possibly add a note on the susceptibility of the red blood corpuscles in various diseases to water containing graduated percentages of sodium chloride.

Normal Blood.

It is stated in a paper published in the 'Proceedings of the Royal Society' by one of us (L. S. D.),* that while auto-agglutination of the red blood corpuscles does not occur, iso-agglutination is met with in a certain proportion of cases. The samples of blood used in these experiments have been obtained from workers in these laboratories and others apparently in the best of health. The technique was similar to that referred to in the preliminary report.†

Some samples of normal sera have the power of agglutinating almost all specimens of normal and abnormal red cells presented to them, and often the agglutination is of the hæmolytic type—blood presenting these changes has been obtained from apparently perfectly healthy men, but in no case was auto-agglutination or auto-hæmolysis met with. The hæm-agglutinating properties of "pooled" serum were tested in the manner about to be recorded. Several samples of sera in equal proportions were allowed to interact at 37° C., and then tested with various suspensions of red blood corpuscles which had previously been treated with the sera individually. The results of these experiments may be briefly summarised. It was found that when a serum caused agglutination of certain red cells, agglutination still occurred in the pooled mixture, but to a less degree owing to dilution; but if no agglutination of the suspension of red blood corpuscles had taken place in any of the individual sera before pooling, no effect was noted when the pooled serum was added to the red cells. In a certain proportion of the samples of normal blood examined for agglutinative properties a negative result was obtained; but some specimens, while not exhibiting true agglutination of the individual chromocytes, showed a definite clumping of rouleaux. The appearance of this type of agglutination was extremely rapid, and could be watched under the microscope immediately after the specimen had been prepared.

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* 'Roy. Soc. Proc.,' B, vol. 81, p. 207.

† 'Roy. Soc. Proc.,' B, vol. 80, p. 531.

In another series of experiments the mixtures of the respective sera and red cells were tested under special conditions of temperature; the blood was collected in citrated saline at 37° C. and kept at that temperature; the cells were washed with saline also at 37° C., and the suspension of red cells was made up in this warm saline, but these precautions did not appear to influence the results in any way.

Undiluted Red Cells.—After the red cells had been washed in saline and the supernatant fluid pipetted off, a mixture of one volume of serum and one volume of undiluted red cells was made. In one series of experiments the 5-per-cent. suspension of red cells gave no reaction with the appropriate serum, but when tested by this method perfect agglutination occurred instantaneously. This experiment was the only instance in which such a phenomenon was noted. In all other cases when negative results were obtained by the 5-per-cent. red-cell suspension, similar results were obtained by using the undiluted red cells.

Hæm-agglutinins.

In the series of cases concerning this investigation no instance of true auto-agglutination has been met with. It has been shown that frequently the serum or red cells of a patient suffering from a disease may agglutinate or be agglutinated by the red cells or serum of a patient suffering from the same disease.

In many instances it was found that, by heating a serum at 60° C. for 15 minutes and thereby destroying its hæmolytic property, the agglutinative action was rendered much more obvious than previously, and this was especially the case in those examples where the haemolytic action was very intense. In one series of experiments on hæm-agglutination neutral fluid was used (0·8 per cent. sodium chloride and 0·02 per cent. calcium chloride), but no advantage appeared to occur by employing a 5-per-cent. suspension of red cells in this fluid.

Further attempts were made to extract hypothetical agglutinins from the red cells themselves. The contents of a tube containing serum and unwashed red cells were pounded in a mortar with fine glass and then allowed to stand all night. The red fluid obtained after centrifugalisation was completed was then tested on samples of red cells which the untreated serum had failed to agglutinate, but negative results occurred in every instance.

Specific Agglutinins.

The technique adopted for these investigations has been similar to that referred to in detail in the previous reports. The experiments connected with specificity have so far been of a confirmatory nature, that is, if a serum is saturated with suitable red cells, the agglutinative property of the serum is removed for those red cells, but remains for other red cells which were agglutinated by the serum previous to saturation, although exceptions very occasionally occur. The following experiment well illustrates this true specific action.

Immune serum (pneumonia) + normal red cells = marked agglutination.

Immune serum (pneumonia) + immune red cells (pernicious anæmia) = marked agglutination.

The immune serum was then saturated with normal red cells for two and a quarter hours at 37° C. and then centrifugalised at high speed.

Resulting clear fluid + normal red cells = *no* agglutination.

Resulting clear fluid + immune red cells (pernicious anæmia) = marked agglutination (as before).

It is interesting to note here that in the series of experiments with normal blood, when marked agglutination of the red cells occurred with the serum of another normal person, similar specificity could definitely be demonstrated.

Heated Red Cells.—In the 'Proceedings of the Royal Society'* a few observations were made as to whether heated red blood corpuscles still retained their specific properties. In these experiments it was found that thoroughly washed red cells heated at 60° C. for one hour were still capable of removing the agglutinative properties of a serum, as was the case with the unheated cells. The technique adopted in these experiments has already been given in detail. In those examples about to be referred to varying ranges of temperature have been employed from 66° C. up to boiling point.

We have found that red cells heated to a temperature of 66° C. for one hour, or at 100° C. for half an hour, are still able to render a suitable serum inactive when carried to the point of saturation.

The following experiment will serve as an illustration:—

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* 'Roy. Soc. Proc.,' B, vol. 81, p. 214.

Immune pneumonic serum when added to normal red cells produced marked agglutination, but when added to the auto-red cells no effect was produced. Two samples of each type of red cell were placed in suitable sealed glass tubes and heated (i) at 95° C. for 30 minutes, (ii) at 63° C. for one hour. The same immune serum was then saturated with these red cells for 12 hours at 37° C., the clear fluid obtained from each mixture after centrifugalisation had been completed was added to normal red cells and the pneumonic red cells as in the experiments previous to saturation; it was now found that the clear fluid obtained after the immune serum had been saturated with the normal red cells at both ranges of temperature failed to agglutinate normal red cells. In another instance red cells which had been exposed to a temperature of 100° C. for 30 minutes were capable of removing the specific agglutinative action of a certain serum, and this result could be shown to be strictly specific.

Dried Red Cells.—It was thought possible that, as red cells which had been exposed to high temperature were still capable of exerting their specific action, similar results might be obtained with dried red cells. The technique adopted in these experiments was as follows:—

Various samples of citrated red blood corpuscles were thoroughly washed in normal saline, and then freed as far as possible from saline. The thick suspensions of the various red cells were placed in watch-glasses and dried in a partial vacuum over chloride of calcium, the entire process of drying lasting some 12 to 24 hours. The dried blood was then pounded in an agate mortar to a fine brick-dust powder; samples of sera were then saturated with these dried and pounded red cells. The technique for the experiments on saturation was similar to that already referred to with the heated red cells, and the results were likewise similar, that is to say, red cells thoroughly dried and pounded were still capable of preserving their specific functions, as this experiment will serve to illustrate.

A mixture of normal serum and jaundiced red cells gave rise to marked agglutination; this serum, when saturated with these dried and pounded red cells, was no longer capable of agglutinating them, but it was still able to agglutinate other samples of red cells which it had been able to do previous to saturation.

Sand.—Since red cells which have been exposed to these high temperatures although so physically altered are still capable of exerting a specific agglutinative action, it was thought that sand heated to a similar temperature might give rise to identical results, but it was found from experimental observations to be inert.

Hæmolysins.

Numerous additional observations have been made in regard to hæmolysins, and, as stated in the previous report, it is only comparatively rarely that a well-marked hæmolytic action is obtained. In those cases in which hæmolysis occurs hæm-agglutination is always present, but the more marked the hæmolysis in certain instances the less marked the hæm-agglutination. In some instances the hæmolytic mixtures were put straight into ice for 1 hour, and then into the water-bath at 37° C. for 1½ hours. In the second report it was stated that "the final results in all cases were similar," but in this series of cases we have found that the effect of this method was to reduce the hæmolytic intensity of the mixture, while, in some cases, the hæmolytic action was completely abolished.

It was thought possible, by heating a serum at 60° C. for 15 to 20 minutes and then fully complementing it with another serum, auto-hæmolysis might be excited, but in no instance did this occur.

Splenic Extract.—Further observations have been made in connection with the hæmolytic action of splenic juice, but all results concerning that subject in this report have been negative. In the case of acute miliary tuberculosis, although the immune serum possessed a high degree of hæmolytic action for normal red cells, yet the splenic juice failed to act. A similar result was also obtained in a fatal case of acute lobar pneumonia. On the other hand, it was pointed out in the second report that the splenic extract obtained from a case of diabetic coma completely hæmolysed normal red cells and the auto-immune red cells, while the immune serum was inert in the latter instance, and was of very limited value in the former.

Jaundice.—Attention has previously been drawn to a case of jaundice in which auto-hæmolysis occurred; further observations, however, have failed to record another example. Additional cases of jaundice from all possible causes have been examined for the purpose of ascertaining whether the presence of bile pigment in the blood serum favours iso-hæmolysis, but no direct connection can be traced. In those instances in which hæmolysis has occurred the cases have been almost entirely due to malignant growths.

The Resistance of the Red Cells to Water containing Sodium Chloride of various Strengths.

H. P. Hawkins and one of us (L. S. D.)* showed that the red cells in two cases of congenital cholæmia were especially susceptible to the action of hypotonic solutions of sodium chloride. These observations have since been confirmed by Hutchison and Panton.* This investigation was undertaken with the idea that the red cells under various pathological conditions would show wide variations in their susceptibility to saline solutions, more especially in such diseases as pernicious anæmia and the severe septic infections.

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* 'The Quarterly Journal of Medicine,' January, 1909, vol. 2, No. 6, p. 165.

Technique.—In all cases a standardised 1-per-cent. solution of sodium chloride was made up in fresh distilled water, and from this solution various strengths of sodium chloride were added to small glass tubes arranged in series, in equal volumes; usually the first tube contained 1 per cent. sodium chloride, and the last 0·1 per cent. The blood was obtained from the patient's thumb under light pressure in the same capillary tube as was employed for making the saline dilutions. Each tube contained, therefore, 10 equal volumes of saline, and one volume of blood amounting to 1/11th of the total. The whole was mixed thoroughly and allowed to stand at room temperature; no advantage was derived either from storing at 37° C. or in ice.

As already stated, most interesting results were obtained in two cases of congenital cholæmia; here hæmolysis occurred in a solution of sodium chloride up to 0·6 per cent.

In one case of virulent erysipelas a similar result was obtained, but otherwise the blood in various pathological conditions showed little variation from the normal, that is to say, hæmolysis was prevented in a mixture containing 0·4 per cent. sodium chloride, and a positive reaction occurred in a 0·3-per-cent. solution.

In all these experiments, with the exceptions referred to, the susceptibility of the red cell fell within the limits of the normal, and no further information could be obtained although the technique was modified in various ways, such as washing the red cells and adding sodium citrate in definite proportions, and working with a neutral fluid.

Phagocytosis.

In this report numerous experiments have been completed concerning bacterial phagocytosis. In the first series of experiments the serum and leucocytes obtained from apparently healthy persons were compared as to their respective activity. In every instance strains of the *Bacillus coli* or the *Staphylococcus aureus* were employed; and the number of leucocytes was approximately the same in the various samples of blood. Other observations were made by comparing the immune serum from different diseases with normal leucocytes obtained from apparently healthy people, and conversely, normal serum was allowed to act in the presence of immune leucocytes obtained from various diseases.

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* 'The Quarterly Journal of Medicine,' July, 1909.

It was thought that the different results which were obtained when the serum from one case was mixed with the red cells and leucocytes of another might be due partly to hæmolysis or to a process of de-opsonisation owing to the mixture of serum with suitable red cells. Robert Muir and Martin, in a paper published in the 'Proceedings of the Royal Society,'* showed that a serum could be largely de-opsonised by saturation with various substances, among them suitable red blood corpuscles. In their experiments suitable red cells and serum were allowed to remain in contact at 37° C. for a considerable period of time.

If the various results obtained by allowing a normal serum to act in the presence of normal leucocytes and red cells, as compared with the immune serum with normal red cells and leucocytes, were partly dependent upon a hæmolytic action, this change would have to take place during a period of 15 to 20 minutes, because this is the time commonly employed for the incubation of phagocytic mixtures. The prolonged periods of time as used in saturation experiments would be unsuitable to explain the different results obtained in so short a period of incubation as 15 to 20 minutes.

In every observation on phagocytosis carried out in these experiments records were also made as to whether hæm-agglutination and hæmolysis occurred.

Attempts were made to ascertain whether it was possible to prove the actual part played by hæmolysis.† The technique employed to elucidate these points was as follows:—

The serum which was being examined for its power of exciting phagocytosis was saturated with the washed red cells belonging to the phagocytic mixture; the whole was placed in glass tubes sealed and incubated at a temperature of 37° C. for 15 minutes. The tubes were then centrifugalised at high speed and the clear fluid was compared with the serum previous to saturation.

Control experiments were conducted with a mixture of serum and red cells in which no agglutination or hæmolysis occurred. The first series of observations conducted along these lines concerns the blood obtained from apparently healthy individuals.

Normal serum (L) + normal leucocytes (R) + *Staphylococcus aureus*,
50 cells = 270 cocci.

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* 'Roy. Soc. Proc.,' B, vol. 79, May, 1907, Robert Muir and Martin.

† It has been found from experimental observations that the highest phagocytic readings occur with specimens of blood in which hæmolysis can be demonstrated, although notable exceptions do occur.

After the serum had been saturated with the red cells in the manner described for 15 minutes at 37° C., the following result was obtained:—

The "serum" + normal leucocytes (R) + *Staphylococcus aureus*,
50 cells = 94 cocci.

In this experiment it was shown that L.'s serum had a powerful hæmolytic action on R.'s red cells, and we find as a result of saturating this serum with these red cells that there is a reduction of phagocytic activity from 270 to 94 in so short a time as 15 minutes. A control experiment conducted with R.'s serum and R.'s leucocytes showed 155 cocci in 50 cells before saturation, and 148 following saturation, while with R.'s serum and D.'s leucocytes 176 cocci were engulfed, and subsequent to saturation 168 cocci—in both these experiments neither hæmolysis nor hæm-agglutination occurred. We have here, therefore, an instance of a certain normal serum which is markedly hæmolytic for normal red blood corpuscles, and in which greatly reduced phagocytosis could be demonstrated by saturating it with these red cells for 15 minutes. It must be pointed out here, however, that in some instances, although hæmolysis occurs, there is no striking decrease in phagocytosis when the serum is saturated with suitable red cells for a period of 15 minutes at 37° C. It can also be stated, as a result of a very large number of observations, that a reduced phagocytosis is entirely related to hæmolysis, and is unaffected by the presence of hæm-agglutinins. Similar results can be shown when an immune serum acts in the presence of normal leucocytes. In the experiment to be recorded the immune serum had a powerful hæmolytic action on the normal red cells.

Immune serum + normal leucocytes + *Staphylococcus aureus*,
50 cells = 388 cocci.

The serum was saturated with the normal red cells for 15 minutes at 37° C., and then centrifugalised at high speed. When the serum, after saturation, was added to the same leucocytes and cocci, 50 cells contained 146 cocci. In a control experiment with the normal serum and normal leucocytes obtained from the same individual, in which there was an absence of hæmolysis, there was only a very slight reduction in phagocytosis.

In the next experiment the immune serum from one case was allowed to act in the presence of immune red cells of another.

Immune serum (A) + immune leucocytes (B) + *B. coli*,
50 cells = 249 bacilli.

Experiment was repeated after the same serum had been saturated in the manner indicated for—

$\frac{1}{4}$ hour at 37° C., 50 cells = 146 bacilli.
1 hour at 37° C., 50 cells = 100 bacilli.

In this instance the immune serum (A) was found to be powerfully hæmolytic on immune red cells (B), but on the other hand the same red cells were hæmolysed by another sample of immune serum, but in this instance there was no marked reduction in phagocytosis after saturation for a quarter of an hour at 37° C.

In a paper published in the 'Proceedings of the Royal Society,'* by Mr. Shattock in conjunction with one of us (L. S. D.), it was shown that leucocytes obtained from different sources may vary in their phagocytic value like the serum. From numerous observations conducted along the same lines by us a large amount of confirmatory evidence has been collected.

A few instances may be cited to illustrate these remarks:—

(*a*) Normal serum + normal leucocytes + *Staphylococcus albus*. 50 cells = 254 cocci.

The same serum, however, when added to immune leucocytes, which were obtained from the patient from whom this identical organism was grown in pure culture, and the *Staphylococcus albus* showed 106 cocci in 50 cells.

While the immune serum obtained from this case, when added to the normal leucocytes referred to above and the cocci, gave 388 cocci in 50 cells.

(*b*) Immune serum of pulmonary tuberculosis acting in the presence of its own leucocytes and cocci showed 174 cocci in 50 leucocytes, but when this immune serum acted in the presence of normal leucocytes and the same staphylococci, 50 cells contained 433 cocci.

(*c*) One more experiment only will be referred to concerning this point.

Normal serum + normal leucocytes + *Bacillus coli*; 50 cells = 220 bacilli, but when the same serum acted in presence of leucocytes from a case of acute lobar pneumonia, 50 cells = 83 bacilli.

In all these experiments on phagocytosis the serum was examined within a few hours of its removal from the body, and was free from blood tinging, and the various experiments were made at about the same period.

Robert Muir, in the paper published in the 'Proceedings of the Royal Society,' already referred to, furnished evidence to show that when a serum was saturated with suitable red cells the normal opsonin was removed. In the experiments which we have brought forward on human blood in the present communication, we have similarly found that when a serum is saturated with suitable red cells its phagocytic value may be considerably reduced, but that this does not occur in a mixture in which hæmolysis is absent. It must be apparent from these results that the most accurate information on phagocytosis must be arrived at when the samples of blood are taken direct from the patient and not mixed with other blood.

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* 'Roy. Soc. Proc.,' B, vol. 80, p. 165.

In the experiments about to be referred to, serum and red cells were employed from the same patient, in which, therefore, no hæmolysis would occur, and serum and red cells from different sources in which hæmolysis could be demonstrated, and lastly serum and unsuitable red cells from different sources in which no hæmolytic activity was evident.

The technique may be briefly described:—The corpuscles were collected in the usual manner in citrated saline and then carefully washed in saline; the surface layers were removed so as to get rid of as many leucocytes as possible, and a thick suspension of red cells was employed. Equal volumes of the washed undiluted red cells and serum were thoroughly ground up in an agate mortar with finely powdered glass. The mixture was then placed in glass tubes, sealed and incubated at 37° C. for one hour; the tubes were centrifugalised at high speed, and a mixture in three layers was thus obtained, the uppermost consisting of bright red serum, the lowermost of tinged glass, and the intermediate of fragmented red cells. Phagocytic experiments were then carried out; the first tube contained untreated serum, leucocytes, and micro-organisms, the second tube the "red" serum, leucocytes, and micro-organisms. In each case the leucocytes were obtained from the same mixture, and belonged to the same specimen of blood as had been employed in the experiments just referred to. The serum before and after treatment was derived from the same source.

It was found, as a result of these experiments, that when a serum was treated in the manner indicated, whether it was mixed with red cells derived from the same source or different, or whether from the red cells which formed a hæmolytic mixture or otherwise, the results were very similar. For example:—

	No. of cells counted.	No. of bacilli in cells.	No. of non-active phagocytes.
Experiment 1—			
Untreated immune serum (A) + immune leucocytes (A) + *B. coli.*	50	177	2
Treated „ „ „ „ „	50	60	10
Experiment 2—			
Untreated immune serum (B) + immune leucocytes (B) + *B. coli.*	50	167	6
Treated „ „ „ „ „	50	5	46
Experiment 3—			
Untreated normal serum (A) + normal leucocytes (A) + *B. coli.*	50	168	5
Treated „ „ „ „ „	50	14	38
Experiment 4*—			
Untreated immune serum (A) + normal leucocytes (A) + *B. coli.*	50	324	4
Treated „ „ „ „	50	37	29
Experiment 5—			
Untreated immune serum (B) + normal leucocytes (A) + *B. coli.*	50	233	1
Treated „ „ „ „ „	50	59	20
Experiment 6—			
Untreated normal serum (A) + immune leucocytes (A) + *B. coli.*	50	157	5
Treated „ „ „ „ „	50	59	18

* Active hæmolysis was demonstrated.

It is evident that these results do not depend on a hæmolysing agent, because in five out of the six experiments neither agglutination nor hæmolysis occurred, and yet the degree of phagocytosis was very considerably reduced. Attention has already been drawn to the reduced phagocytosis occurring as a result of hæmolysis, but from these experiments it has been shown that a similar result may be obtained by thoroughly grinding red cells in serum from the same individual and from other sources apart from true hæmolysis.

This action may depend upon the removal from the serum of incitor substances, or by the addition to the serum of specific substances derived from the red blood corpuscles, or lastly through non-specific substances common to all red cells. Further experimental observations are necessary to prove what these results are dependent upon.

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Page

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THE ACCUMULATION OF HELIUM IN GEOLOGICAL TIME.—II. By the Hon. R. J. Strutt, F.R.S. 96

THE ABERRATIONS OF A SYMMETRICAL OPTICAL INSTRUMENT. By H. C. Pocklington, M.A., D.Sc., F.R.S. 99

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THE MOTIONAL EFFECTS OF THE MAXWELL ÆTHER-STRESS. By E. Cunningham, M.A. 109

ON THE CHANGE IN HUE OF SPECTRUM COLOURS BY DILUTION WITH WHITE LIGHT. By Sir W. de W. Abney, K.C.B., D.Sc., D.C.L., F.R.S. 120

HARMONIC TIDAL CONSTANTS FOR CERTAIN CHINESE AND NEW ZEALAND PORTS. By Thomas Wright.. 127

THE ELECTRIC CONDUCTIVITY AND DENSITY OF SOLUTIONS OF HYDROGEN FLUORIDE. By Prof. E. G. Hill, D.Sc., and Dr. A. P. Sirkar 130

OBITUARY NOTICES OF FELLOWS DECEASED: Eleuthère Élie Nicolas Mascart; William Edward Wilson; Francis Elgar; Georg von Neumayer; Lord Rosse; Antoine Henri Becquerel .. .. i—xxiii

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CONTENTS.

Page

The Germicidal Action of Metals and its Relation to the Production of Peroxide of Hydrogen. By ALLAN C. RANKIN, M.D., Demonstrator in Bacteriology, McGill University, and Assistant in Bacteriology, Royal Victoria Hospital, Montreal 78

Gametogenesis of the Gall-Fly, Neuroterus lenticularis (Spathegaster baccarum).—Part I. By LEONARD DONCASTER, M.A., Lecturer on Zoology, Birmingham University. (Plates 1—3) 88

Preliminary Note upon the Cell Lamination of the Cerebral Cortex of Echidna, with an Enumeration of the Fibres in the Cranial Nerves. By EDGAR SCHUSTER, D.Sc., Fellow of New College (Pathological Laboratory, Claybury Asylum, Essex, and Department of Comparative Anatomy, University Museum, Oxford). (Plates 4 and 5) 113

Cortical Lamination and Localisation in the Brain of the Marmoset. By F. W. MOTT, M.D., F.R.S.; E. SCHUSTER, D.Sc.; and W. D. HALLIBURTON, M.D., F.R.S. (Plates 6 and 7) 124

The Caudal Fin of Fishes (Preliminary Paper). By RICHARD H. WHITEHOUSE, M.Sc. Birm. 134

Some Experiments with the Venom of Causus rhombeatus. By H. E. ARBUCKLE, M.B., West African Medical Service 144

On the Comparative Action of Stovaine and Cocaine as measured by their Direct Effect upon the Contractility of Isolated Muscle. By V. H. VELEY, F.R.S., and A. D. WALLER, F.R.S. 147

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	No. of cells counted.	No. of bacilli in cells.	No. of non-active phagocytes.
Experiment 1—			
Untreated immune serum (A) + immune leucocytes (A) + *B. coli.*	50	177	2
Treated ,, ,, ,, ,, ,,	50	60	10
Experiment 2—			
Untreated immune serum (B) + immune leucocytes (B) + *B. coli.*	50	167	6
Treated ,, ,, ,, ,, ,,	50	5	46
Experiment 3—			
Untreated normal serum (A) + normal leucocytes (A) + *B. coli.*	50	168	5
Treated ,, ,, ,, ,, ,,	50	14	38
Experiment 4*—			
Untreated immune serum (A) + normal leucocytes (A) + *B. coli.*	50	324	4
Treated ,, ,, ,, ,, ,,	50	37	29
Experiment 5—			
Untreated immune serum (B) + normal leucocytes (A) + *B. coli.*	50	233	1
Treated ,, ,, ,, ,, ,,	50	59	20
Experiment 6—			
Untreated normal serum (A) + immune leucocytes (A) + *B. coli.*	50	157	5
Treated ,, ,, ,, ,, ,,	50	59	18

* Active hæmolysis was demonstrated.

It is evident that these results do not depend on a hæmolysing agent, because in five out of the six experiments neither agglutination nor hæmolysis occurred, and yet the degree of phagocytosis was very considerably reduced. Attention has already been drawn to the reduced phagocytosis occurring as a result of hæmolysis, but from these experiments it has been shown that a similar result may be obtained by thoroughly grinding red cells in serum from the same individual and from other sources apart from true hæmolysis.

This action may depend upon the removal from the serum of incitor substances, or by the addition to the serum of specific substances derived from the red blood corpuscles, or lastly through non-specific substances common to all red cells. Further experimental observations are necessary to prove what these results are dependent upon.

The Germicidal Action of Metals and its Relation to the Production of Peroxide of Hydrogen.

By Allan C. Rankin, M.D., Demonstrator in Bacteriology, McGill University, and Assistant in Bacteriology, Royal Victoria Hospital, Montreal.

(Communicated by J. G. Adami, F.R.S. Received June 12,—Read June 24, 1909.)

(From the Pathological Laboratory, Royal Victoria Hospital.)

That sundry metals possess not merely a distinct inhibitory action upon the growth of moulds, bacteria, and other micro-organisms, but even possess germicidal properties has been known for a long period, and there have been numerous observations demonstrating this action. The earliest observation known to us is that by Raulin in the seventies, upon the remarkable inhibition produced by traces of silver upon the growth of the *Aspergillus niger*. More recent work has in general followed the lines of the experiment employed by Miller, in his observations upon the bactericidal properties of gold and various alloys employed in dentistry; namely, plate cultures have been made of various micro-organisms, and into the inoculated culture media, while still fluid, small pieces or plates of the metals have been dropped, the presence or absence of a clear surrounding zone indicating arrest of growth of inoculated bacteria. As a result of these observations, it has been shown that certain metals possess inhibitory powers. More than this, according to Behring, reinoculation of the clear zone after the removal of the metal may lead to continued negative results. This would indicate that not merely is there inhibition, but an alteration of the gelatin, and suggests strongly the presence there of substances having a distinct germicidal effect. A full study of this nature was made by Bolton. Other observations (Ficker, Von der Dois, Clarke and Gage) have been along the lines of either employing metal vessels to hold the inoculated fluids, or placing strips of the metal in glass vessels holding water containing known cultures of bacteria. In these experiments also, metals like copper, zinc, and silver have been noted to possess distinct bactericidal effects. But in the above observations there has been no adequate attempt to determine the means whereby the metals exert their bactericidal action. Leedham-Green, however, made the observation that iron was inert or active according as to whether the conditions of the experiment prevented or permitted free oxidation of the metal. It was through oxidation that inhibitory action was brought about. The general opinion has been that there is a diffusion of the metal into the water or other medium, and that in some direct way the metal arrests the growth or actually brings about the death of the micro-organisms.

Certain observations made by Prof. Barnes, and a recent paper by Barnes and Shearer, have opened up the possibility of gaining a more direct knowledge of the mechanism by which these metals act, and the present series of observations owes its origin to Prof. Barnes. Barnes and Shearer working with aluminium showed experimentally that aluminium foil in water containing dissolved oxygen generated peroxide of hydrogen according to the following hypothetical equation:—

$$2Al + 6H_2O + 6O = Al_2(OH)_6 + 3H_2O_2.$$

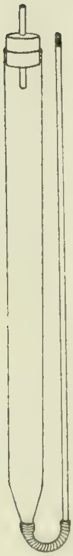
Concerning the production of the peroxide they write as follows:—"We placed pure aluminium sheet in ordinary pure distilled water open to the air and thoroughly charged, and after standing for a few hours we applied the well-known potassium iodide test for peroxide with marked success. Having obtained evidence of the production of the peroxide, we found that the yield could be increased by using considerable quantities of aluminium foil in small pieces, and leaving it for two or three days in water, through which air bubbled." They further showed that the production of peroxide of hydrogen depended upon the presence of dissolved oxygen, and subsequently that zinc acted in a manner similar to aluminium, and that with copper there was no production of peroxide of hydrogen.

The germicidal action of hydrogen peroxide is well known. It became, however, a matter of interest to observe in the first place in connection with those metals which in water gave origin to hydrogen peroxide, how far their bactericidal properties bore relationship to the presence of the peroxide, and, in connection with the metals causing no development of peroxide, whether any cause could be determined for their activity. The problems to be solved demanded the development of a series of experiments: in the first place, upon the maximal production of hydrogen peroxide through the agency of the pure metals, and later the effects of the metal in water containing dissolved oxygen, and lastly in oxygen-free water.

After some preliminary investigation during which many experiments were performed, the technique to be described was adopted, and, although some of the results here made use of were obtained prior to the improved method of investigation generally adopted, subsequent experiments showed them to be sufficiently accurate as compared with the newer method, the results varying slightly in degree, but not in kind. The metals used in the experiments were pure and were supplied by Prof. Barnes. The water used was the Montreal tap water, which is a soft alkaline water derived from the Ottawa River, containing variable, but in general small numbers of natural micro-organisms.

The medium used was plain nutrient agar, and the plates were incubated at 37° C. In the later experiments a medium containing iron, esculin and taurocholate of sodium, such as has recently been described by Prof. Harrison, was used. *B. coli* were added to the water drawn from the tap, so that the number of organisms very greatly exceeded that found usually in contaminated water.

A tube 40 cm. long by 3·5 cm. in diameter was drawn out to a nipple at one end. Over this was fitted a piece of rubber tubing with a section of glass tubing fitted on to the rubber at its distal end (see figure). The rubber was of such length that it was possible to attach the smaller tube to the side of the large one. The small tube extended above the level of the large one, and was, when sterile, plugged with cotton wool. The whole was then relatively sterilised by passing water at 70° C. through it for one hour. The metal having been previously cleaned and thoroughly washed in running water was dropped into the tube by the unsterile hand; 155 c.c. of the inoculated tap water was then added, and the apparatus attached to a Sprengel pump, and air bubbled through the water for a period of one hour. At the end of that time the rubber at the base of the large tube was pinched with the fingers, the attachment to the Sprengel pump disconnected, and a quantity of water removed from the larger tube by means of a sterile pipette, plated on agar, 1/20 c.c. in each case, and incubated at 37° C. for 24 hours, when the plates were examined and compared with plates to which a similar quantity of the untreated inoculated water had been previously added. In the tables these are referred to as Plate 2 and Plate 1 respectively. For determining the amount of peroxide of hydrogen present Bach's test was employed, as well as the potassium iodide test.



Control Experiment.—Using the above technique, inoculated water, containing *B. coli*, was bubbled for a period of one hour without the addition of any metal. There was slight, if any, reduction in the number of organisms in the water. The simple bubbling of air for one hour through water containing *B. coli*, then, does not cause any noticeable reduction in the number of organisms present in the water.

Effects of Metal without "Bubbling."—The following experiments were performed to see what effects metallic aluminium, zinc, and copper had upon bacteria when placed in contact with water at rest containing *B. coli* in the apparatus described. The results are shown below. It will be observed that zinc exerts some effect upon *B. coli* in one hour. It is noteworthy that aluminium and copper exerted none:—

Metal.		Water.	Time.	1st plate, 1/20 c.c.	2nd plate, 1/20 c.c.	Hydrogen peroxide.
		c.c.	hour.			
Aluminium ...	50 grammes	150	1	Innumerable*	Innumerable	No reaction.
Zinc	50 „	150	1	„	Slight reduction	„
Copper	50 „	150	1	„	Innumerable	„

* At least 7000 to 8000 colonies per 0·05 c.c.

The water used in these experiments was obtained from the tap and held a fair amount of oxygen in solution. It is seen from this experiment that there is a bactericidal action (in the case of zinc) without demonstrable production of peroxide of hydrogen. In studying this it seemed possible that the oxygen in solution in the water played, nevertheless, some part in the destruction of the organisms, presumably by interaction with the metal.

Effects of Oxygen-free Water.—To investigate this the following experiments were performed. Tap water was again used, but previous to placing it in contact with the metals it was boiled for half an hour and then cooled:—

Metal.		Water.	Time.	1st plate, 1/20 c.c.	2nd plate, 1/20 c.c.	Hydrogen peroxide.
		c.c.	hour.			
Aluminium ...	50 grammes	180	1	Innumerable	Innumerable	None.
Zinc	50 „	180	1	„	„	„
Copper	50 „	180	1	„	„	„

Here we see that there is practically no reduction with any of the metals.

Effects of Bubbling of Air on the Solubility of the Metal.—In the next experiment water was added to the three metals and air was drawn through the apparatus for a period of one hour, and subsequently the water was tested quantitatively for metal in solution.

Metal.		Water.	Metal in solution.
		c.c.	
Aluminium.........	50 grammes	150	Trace—hydrate thrown down.
Zinc..................	50 „	150	„ „ „
Copper	50 „	150	Less than 0·0015 part per 100,000.

It can hardly be possible that this small quantity of metal in solution can be responsible for any germicidal action exhibited in the experiments to follow. The work of Clarke and Gage would also point to the fact that it was not so. To investigate the point, however, the following experiment was performed:—

Aluminium, zinc, and copper were bubbled for a period of one hour in tap water, and the water was then withdrawn into a sterile bottle and allowed to stand overnight. These three portions of water were then inoculated with *B. coli;* plates were made immediately, and again in one hour. The results are given below:—

Metal.	Water.	Time.	1st plate, 1/20 c.c.	2nd plate, 1/20 c.c.
	c.c.	hour.		
Aluminium... 50 grammes ...	150	1	Innumerable	Innumerable.
Zinc............ 50 ,, ...	150	1	,,	,,
Copper ...... 50 ,, ...	150	1	,,	Slight reduction.

These results conform to the previous experiments, and were to be expected. Others have shown that small quantities of copper may at first inhibit the growth of *B. coli*, but that subsequently the results are very similar to those obtained when *B. coli* are added to sterile water in glass vessels and allowed to stand. The results with aluminium and zinc show that the amounts of these metals in solution exert no bactericidal action; that the small quantity of peroxide of hydrogen still present exerts no effective action.

Production of Peroxide of Hydrogen.—Quantitative tests of the amount of *available oxygen* in the water after air had been bubbled through for one hour gave the following figures:—

With zinc—

(1)............... 0·318 per cent. by volume, equivalent to 0·00096 per cent. hydrogen peroxide by weight.

(2)............... 0·299 per cent. by volume, equivalent to 0·00090 per cent. hydrogen peroxide by weight.

With aluminium—

(1)............... 0·252 per cent. by volume, equivalent to 0·00076 per cent. hydrogen pexoxide by weight.

(2)............... 0·195 per cent. by volume, equivalent to 0·00059 per cent. hydrogen peroxide by weight.

With copper......... None.

I do not need to emphasise the point that these amounts of peroxide per cent. can have little germicidal effect. Obviously the results to be noted below are not due to the mere presence of peroxide, but are due to some action with which is associated the production of the peroxide.

Germicidal Effect of Aluminium.—Aluminium strip cut into small squares and cleaned was then experimented with. The metal, in various proportions by weight, was bubbled with water containing *B. coli.* The results are shown below :—

Quantity of metal.	Water.	Time.	1st plate, 1/20 c.c.	2nd plate, 1/20 c.c.	Peroxide.
	c.c.	hour.			
Aluminium, 125 grammes	150	1	Innumerable	1 colony	Present.
,, 100 ,,	150	1	,,	5 colonies	,,
,, 75 ,,	150	1	,,	1 in 48 hrs.	,,
,, 50 ,,	150	1	,,	1	,,
,, 25 ,,	150	1	,,	Many	Slight trace.
,, 25 ,,	150	1	,,	,,	,,

The first two experiments in the series were performed prior to a change in technique, and it was subsequently shown that 50 grammes of aluminium caused, depending upon the cleanliness of the metal, a good production of peroxide of hydrogen, and would generally give a plate free from colonies. A fair amount of peroxide of hydrogen is formed and a hydrate is thrown down. The hydrate has no opportunity of sedimenting, the bubbles of air keeping it constantly in motion and thoroughly mixed with the water. In taking the test water, therefore, the hydrate is thoroughly mixed with the water, thus excluding the possibility of live organisms being entangled in the precipitate and carried down with it, and thus not appearing on the plates. Quantitative tests for aluminium in solution showed only traces of the metal present.

Germicidal Effects of Zinc.—A series of experiments similar to those performed with aluminium were carried out with granulated zinc. The

Metal.	Water.	Time.	1st plate, 1/20 c.c.	2nd plate, 1/20 c.c.	Peroxide.
	c.c.	hour.		hours.	
Zinc...... 125 grammes	150	1	Innumerable	24 none	
				48 a few	Strong.
,, ... 100 ,,	150	1	,,	24 none	
				48 one	,,
,, ... 75 ,,	150	1	,,	24 none	
				48 three	Fair.
,, ... 50 ,,	150	1	,,	24 none	
				48 two	,,
,, ... 25 ,,	150	1	,,	24 six	
				48 twenty-four	Slight.
,, ... 10 ,,	150	1	,,	24 thirty	,,

peroxide reaction was stronger than with aluminium, and the metal exhibited a more pronounced germicidal action.

With 50 grammes of zinc the plate was usually free from colonies, although occasionally a few colonies would develop. It was noticeable again that if the surface of the metal was not clean the yield of peroxide was slight and the reduction of the organisms in the water not so marked.

Germicidal Effects of Copper.—Pure sheet copper was then substituted for zinc. The following experiment sets forth the germicidal action of copper in this method of experimentation. It will be seen that copper exhibits quite a marked bactericidal effect, and that *without the production of peroxide of hydrogen.* Small quantities of copper could be detected in the water in about 0·0015 part per 100,000.

Metal.	Water.	Time.	Plate before, 1/20 c.c.	Plate after, 1/20 c.c.	Peroxide of hydrogen.
	c.c.	hour.			
Copper...... 125 grammes	150	1	Innumerable	None	None.
„ ...... 125 „	150	1	„	„	„
„ ...... 125 „	150	1	„	„	„
„ ...... 100 „	150	1	„	„	„
„ ...... 75 „	150	1	„	„	„
„ ...... 50 „	150	1	„	30	„
„ ...... 50 „	150	1	„	33	„
„ ...... 25 „	150	1	„	Numerous	„
„ ...... 10 „	150	1	„	„	„

By reference to this table, it will be seen that 50 grammes of copper is not as strong a germicide as a similar quantity of zinc. Nevertheless, under the conditions of the experiment, it exhibits a strong germicidal action. This, as already noted, is: (1) in the absence of production of peroxide of hydrogen, and (2) under the conditions in which the amount of copper passing into solution is inadequate to explain the effects. We further have determined (3) that copper in tap water, with a free supply of oxygen, exhibits a stronger germicidal action than does ordinary tap or boiled water, and also (4) that when water is treated in the same manner, and for the same length of time, without copper being present, and is inoculated with *B. coli* in similar proportions, there is little, if any, reduction of the number of *B. coli* in the water.

Little attempt has been made in the past to associate the bactericidal influence of metals with oxidation or other effects produced by those metals, We learn that Novy and Hendry have carried out certain studies upon the production of "organic peroxides" by metals, and the effect of the same upon bacteria, but although some of their results were communicated verbally to a meeting of the Association of American Bacteriologists there has been no publication, nor am I acquainted with their findings. The only observation known to me is that by Leedham-Green already noted, upon the negative effects of unoxidised iron and the arrest of growth of bacteria when the iron is allowed to undergo oxidation. The general conception has been that the metal undergoes solution, and in this condition is directly active upon the bacteria.

As bearing upon the mode of action of metals, the results of the present investigations may be briefly summed up.

(i) Air drawn for one hour through water containing abundant colon bacilli exercises no perceptible inhibitory action upon their subsequent growth.

(ii) Relatively large amounts of pure zinc with large surface area placed in water, contaminated with abundant colon bacilli, and allowed to act for one hour, bring about a recognisable but not extreme destruction of the bacteria. Aluminium and copper, under similar circumstances, have no perceptible effect.

(iii) When the same experiment is repeated, with the one difference that the oxygen has been driven out of the water by previous boiling, none of these metals has any decided influence upon the subsequent growth of the bacteria.

It is thus obvious that such bactericidal activity of zinc as manifests itself is associated with the coincident presence of oxygen.

(iv) A much more intense bactericidal action is produced when air is permitted to bubble for one hour through water holding the colon bacilli in suspension, in the presence of aluminium, zinc and copper. Using a sufficiency of the pure metal it is possible to render the water completely sterile with all three metals, and that, when it contains abundant bacteria.

(v) In the case of zinc and aluminium, the sterilisation process is accompanied by the production of easily recognisable amounts of peroxide of hydrogen, and formation of hydrates of the two metals.

(vi) While this is the case, the peroxide itself cannot be regarded as the sterilising agent, and this because :—

(*a*) The amount of peroxide developed in one hour by bubbling air through the like quantity of sterile water in the presence of zinc or aluminium, while easily recognisable, is nevertheless very small in amount; a similar dilution of the peroxide added to a suspension of colon bacilli has no perceptible effect.

(*b*) Bactericidal action of the same or greater intensity is exerted by pure copper under like conditions of experiment, and *this with no associated recognisable production of hydrogen peroxide.*

(vii) It is thus evident (*a*) that oxygen must be present in order that these three metals, zinc, aluminium, and copper, may manifest bactericidal properties (in Montreal tap water), and (*b*) that in the process of interaction between the oxygen and the first two of these metals hydrogen peroxide becomes developed, whereas it is not detected in the case of the third.

Are we justified in drawing any conclusions as to the essential nature of the process of bacterial destruction that occurs in the presence of these—and possibly of other—metals? I am inclined to think that our general conception of the mode of production, and, it may be added, of the mode of disinfectant and sterilising action of hydrogen peroxide supplies the clue. We may lay down that the molecule of oxygen coming into contact with any of these three metals becomes dissociated with the liberation of free ions, of which, in the case of zinc and aluminium, some combine with the molecule of water to form the peroxide, and that it is these free ions, or in more old-fashioned language, "nascent oxygen," that is the essential agent in the bactericidal process. It is in this way that we explain the bactericidal activity of peroxide of hydrogen (for although I have laid down that in these particular experiments the amount of hydrogen peroxide developed was inadequate to explain the marked destruction of the bacteria, it must be remembered that this compound has notable disinfectant powers). We believe that the molecule of the peroxide coming into contact with the bacteria becomes dissociated, and that the liberated ion of oxygen is the destructive agent. It is quite possible that the hydrogen peroxide formed is thus a subsidiary agent in the bactericidal process where zinc and aluminium are present. It seems, however, simpler to presume that the dissociation of the oxygen molecule in the presence of water is the feature common to all three metals, and that in the case of the zinc and aluminium experiments the free ions of oxygen act upon the bacteria prior to, or, more accurately, in preference to, becoming associated with the molecules of water to form the peroxide, a certain excess, however, undergoing the latter combination. With regard to copper it must be postulated that the greater affinity of the free ions to the copper molecules than to those of water leads to non-formation of the peroxide or to rapid dissociation of such molecules as may become formed.

Why copper and zinc have diverse actions in the matter of the production of hydrogen peroxide I do not as a bacteriologist pretend to explain; I can but call attention to the facts.

To Prof. Adami my sincere thanks are due for suggestions and advice, as also to Dr. Oskar Klotz for his generous assistance, and to Dr. A. A. Bruere for aid given in the chemical analysis.

NOTE.—Subsequent to the above experiments, further analyses have been made, using boiled *distilled* water in the place of tap water. It was found that zinc in contact with distilled water, without the bubbling process, has a decided germicidal action. Copper has a variable effect, never as strong as that exhibited in the foregoing experiments. Aluminium usually corresponds in effect to that exhibited by plain boiled distilled water, which causes, in an hour, slight reduction in the number of organisms.

Away from the metal—that is, in water which has been in contact with the metal for one hour and then placed in a clean vessel—zinc water (distilled) still causes a reduction in the number of organisms. It will be seen that this is in line with the observations of Behring noted in our introductory paragraph. It is difficult to ascribe this to anything but the direct effect of the metal in solution, and yet, only the minutest traces of the metal are present, quantities which in tap water could hardly be responsible for the effects produced, and indeed would not produce them.

As bearing upon these somewhat divergent results it may be recalled, as a matter of familiar knowledge, that the action of distilled and of acid waters upon copper and other metals differs from that of the (usually) alkaline tap waters.

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Gametogenesis of the Gall-Fly, Neuroterus lenticularis (Spathegaster baccarum).—Part I.

By Leonard Doncaster, M.A., Lecturer on Zoology, Birmingham University.

(Communicated by Prof. W. Bateson, F.R.S. Received September 21,—
Read December 9, 1909.)

[Plates 1—3.]

In previous papers* I have described the maturation of the egg, with some observations on the spermatogenesis, of the Saw-fly, *Nematus ribesii,* in which the eggs may develop either parthenogenetically or after fertilisation. Some questions remained obscure or doubtful, and it seemed probable that an answer to them might be found in a study of one of the gall-flies (Cynipidæ). The gall-flies are remarkable in having in most species two generations in the year, of which one is bisexual, and the other consists wholly of females. The flies of the two generations are, further, so different from one another in structure that in most species they were originally described as belonging to different genera. The galls produced by the two generations are also very distinct.

The species chosen for this work was the very common *Neuroterus lenticularis,* of which the summer (bisexual) generation was originally described as *Spathegaster baccarum.* The galls from which the spring (agamic) generation emerge are lenticular growths found on the underside of oak leaves in October. From these galls the flies hatch early in April; they are exclusively females, and, if provided with buds of oak, readily lay their eggs deep down among the developing leaves and catkins. The eggs were dissected out, fixed in Petrunkewitsch's mixture (alcohol-acetic-sublimate) at various times after being laid, and cut into sections.

The galls of the summer form are spherical, sappy galls, found on the leaves and catkins in May and June. From them hatch males and females (the latter in nature largely preponderating in number), and after copulation the females deposit their eggs in the tissue of young leaves, always at the side of a small vein. The females of this generation differ considerably from the agamic females of the spring brood. The most conspicuous difference is in the ovipositor, which in the summer females is quite short, but in the agamic females is fully five times as long, and coiled up in the abdomen. The flies lay readily, and the eggs may be seen with a lens, so that small pieces of the leaf containing them are easily cut out and fixed. Petrunkewitsch's solution was used, Flemming and others that were tried proving unsatisfactory.

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* 'Q.J.M.S.,' vol. 49, 1906, p. 561, and vol. 51, 1907, p. 101.

For spermatogenesis and the development of the egg before deposition the larvæ and pupæ were removed from the galls at the end of May and early in June, opened and fixed immediately in Flemming (strong) and Petrunkewitsch. For this work Flemming's solution proved the better, but both fixatives gave good results. The chief stain used in all cases was Heidenhain's iron-hæmatoxylin, safranin being used as a control.

Since both males and females arise from the parthenogenetic eggs of the spring generation, it seemed desirable to determine whether both sexes arise from eggs laid by one female, and whether there are any differences in the maturation of the eggs corresponding to the difference in sex. With these objects in view, in collecting and preserving the eggs, in 1909 I kept those laid by individual females separate; and I also made some experiments in letting the flies lay on growing oak branches, and rearing the galls. In April I put single females in muslin bags or "sleeves" on the branches of an oak tree; removed the sleeves and marked the branches when the flies had died, and searched the leaves for galls at the beginning of June. In 1908 these experiments were wholly unsuccessful, perhaps because the tree chosen had not suitable buds for the flies to lay in, for in 1909 I found that they laid much more readily in buds from some trees than from others. The more advanced buds were preferred. In 1909 I sleeved out 40 flies on several different trees, including some quite young oaks from 1 to 4 feet in height. On June 1, I found galls on seven branches of two large oaks, but none on any of the small ones. The numbers of galls in the sleeved branches were respectively 6, 7, 7, 2, 4, 3, 3, and from these I reared respectively 5 ♀'s, 7 ♂'s, 6 ♂'s, 2 ♀'s, 4 ♀'s, 0, 0.

In the galls which did not hatch the larvæ either died or were parasitised.

I searched the trees carefully for galls on branches which had not been sleeved, and found none, so it may be concluded with confidence that these galls were produced by the flies sleeved out. Since in each case only one sex emerged from the galls produced by one fly, although the numbers are small I think it is justifiable to conclude that every fly of the agamic generation produces eggs of only one sex, *i.e.* gives origin to either males or females only, not to both. Cases of this kind are known in the Hemiptera (Aphididæ) and in Rotifers, but not hitherto, as far as I am aware, in the Hymenoptera.

The Somatic Mitoses.

In attempting to count the chromosomes in the mitoses in the egg, it was found difficult to determine the exact number, and with a view to checking this a number of counts were made of division-figures in the body tissues (developing wings, hypodermis) of male and female pupæ. Some of these mitotic figures are represented in Plate 1, figs. 1 and 2. In all cases the chromosomes are nearly equal in size, and the number is certainly approximately 20 in somatic mitoses. In mitoses in male pupæ or larvæ just before pupation, 50 counts gave an average of 19·2 chromosomes, 17 showing 20, and the remainder 19 or 18. In female pupæ of the summer generation, 40 counts gave an average of 19·4, 25 showing 20, and the rest 19 or 18. In the pupæ (female) of the spring generation, division-figures are scarce, as development proceeds much less quickly, but it is clear that the number is sensibly the same as in the summer brood (Plate 1, fig. 3).

The fact that in 50 counts of male mitoses only 17 showed 20 chromosomes, while 25 out of 40 had that number visible in females, might suggest the possibility that the male actually has one chromosome less than the female. In cases such as this, where the chromosomes are small and crowded, it is quite possible to count one elongated or bent chromosome as two, but some of the mitoses with 20 are so clear as to leave no doubt that this is the true number. The difference probably depends on the fact that the female pupa from which most of the counts were made had exceptionally fine mitoses, and was very well preserved, so as to give a smaller proportion of irregular figures than in the male pupæ used. It is possible that in the male one chromosome is smaller than the rest, and sometimes hidden, but I have not been able to convince myself of this.

Spermatogenesis.

The larvæ are found in the galls in the latter half of May; they develop very rapidly, for at the middle of the month they are exceedingly small, and by the end most of the galls contain pupæ. In very young pupæ, with no colour except in the eyes, the testes contain spermatocytes and the various maturation phases; a week later all the pupæ are grey or black, and the "meiotic phase" is over, only spermatids and nearly ripe spermatozoa being found. The full-grown larvæ also have spermatocytes in the testes, and some follicles show prophases of the maturation; for the spermatogonial stages, half-grown larvæ, about four or five days younger, are required. In very young larvæ I have not been able to distinguish the gonads.

The larvæ and pupæ were opened with needles and fixed entire in

Flemming's strong fluid, or sometimes in Petrunkewitsch's fluid; owing to their small size it was found that when the animals were well opened, fixation was quite successful after half an hour in strong Flemming, and if they remained too long the cells became over-fixed. The sections were stained in Heidenhain's iron-hæmatoxylin and sometimes in safranin as a control.

In larvæ, shortly before pupation, the testes contain only spermatocytes, and no division-figures are found. In younger larvæ it is not quite easy to distinguish the sexes, but the ovaries are larger, the cells are beginning to arrange themselves in strands to form the egg-tubes, and some cells are larger than the rest, with very large nuclei, and presumably are developing into eggs. The testes are smaller and all the cells appear alike. They have vesicular nuclei with a large nucleolus, and are much like the embryonic cells of the body-tissues. I have found only one larva* which shows the spermatogonial divisions, and these are remarkable from the large size of the chromosomes and spindle, and from the fact that there are clearly 10 chromosomes at each end in the anaphase (Plate 1, fig. 4, *a*, *b*, *c*). The chromosomes are elongated and rod-like, and when the spindle is cut across, the number 10 can be counted with great confidence at each end. In the same larva mitoses in the body-cells show clearly the diploid number (about 20); in this particular larva I have found none in which I can count the chromosomes with perfect accuracy, but in many mitoses at least 18 can be seen without any doubt, both in metaphase and anaphase. It appears, then, that while the germ-cells contain only 10 chromosomes at this stage, the body-cells contain 20. In female pupæ of the same age, as will be described below, 20 chromosomes appear both in oogonial and somatic divisions.

In larvæ, shortly before pupation, the testes are like those of young pupæ, but contain only primary spermatocytes; in some follicles these are in the resting condition, but in others chromatin is beginning to appear, and where it is aggregated into distinct masses the number of these approaches 20, but they are too irregular for an accurate count to be possible.

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* Since the paper was sent for publication, I have found a second young larva showing 10 chromosomes in the spermatogonial mitoses both in metaphase and anaphase. I have also discovered that in the developing central nervous system of male larvæ (several different specimens) many, but not certainly all, of the mitotic figures show the haploid number of chromosomes (10). In female larvæ the mitoses of the nervous system contain the diploid number. I have not found any perfectly clear equatorial plate in the male nervous tissue showing the diploid number, but some figures suggest it, and it is possible that the supporting cells, like those of the hypodermis, have the full number, and the nerve-cells the reduced. It should also be mentioned that in both sexes there occur in places, in or just below the hypodermis, giant nuclei some 15 μ or 20 μ in diameter, and in one female larva I have found a division figure of one of these nuclei in anaphase with at least 50 chromosomes at each pole. A fuller description, with figures, will be given in the second part of this paper.—*November*, 1909.

The testes in the young pupæ are divided into follicles, in the walls of which are very large nuclei with many chromatin granules. In each follicle the cells are nearly, but not quite, exactly at the same stage of development. This fact makes it very difficult to determine with certainty the exact course of events, for only here and there does one find a follicle in which slightly different stages are represented, and so get a clue as to the course of development. In pupæ which are not too far advanced, various stages occur in different follicles of the same testis from primary spermatocytes before the maturation divisions have begun, up to spermatids which are beginning their metamorphosis into spermatozoa. By comparing a number of testes of this age with one another, I have found a continuous series of stages, and think there is little doubt that in the account which follows they are placed in the correct order. The difficulty is increased by the fact that stages which appear closely similar occur at considerable intervals, and great care is needed in distinguishing one from the other.

The primary spermatocytes are rounded cells lying free in the cavity of the follicle; each has a large vesicular nucleus, containing a very faintly-staining reticulum, and often a nucleolus (Plate 1, fig. 5 *a*, *b*). At the approach of the "meiotic" divisions chromatin appears as small dots close to the nuclear membrane; these dots coalesce into irregular masses just inside the membrane, and these then form themselves into 10 chromosomes, which in some cases for a short time have an elongated band-like form (Plate 1, fig. 6). The cell is meanwhile becoming elongated and pear-shaped, the nucleus occupying the wider end. At the apex of the narrow end a minute black dot, the centrosome (centriole), may often be seen, and at a slightly later stage a second centrosome is seen at the broad end of the cell, close to the nucleus (fig. 7 *a*). I have not observed their origin. In the primary spermatocyte, when the chromatin is beginning to appear, a slightly larger black dot appears outside the nucleus, but as this may still be seen, in addition to the centrosomes, at the stage described, it cannot give origin to the centrosomes.

The chromosomes now become shorter, and place themselves in a radial arrangement, as in an equatorial plate; the nuclear membrane persists, but the nucleus is drawn out towards the narrow apex of the cell, so that it also becomes somewhat pear-shaped (Plate 1, fig. 7 *c*). At this stage, in some cells the narrow apex of the cell is elongated into a fine process, with the centrosome (centriole) at its tip (fig. 7 *c*), like that figured by Mark and

Copeland in the corresponding stage in the Bee.* Fine threads run down from this to the nucleus, but it is difficult to determine whether they penetrate inside the membrane or pass outside it, for at the narrow end the nuclear membrane becomes indistinct and confused with these fibres, while remaining clearly defined at the opposite wider pole.

The stage now described resembles the metaphase of a true division, such as occurs later, but is distinguished from it by the persistent nuclear membrane, and the position of the chromosomes at one end of the nucleus, near the broad end of the cell. In this case no nuclear division takes place, the nucleus becomes oval in shape, and the chromosomes generally contract to form a compact mass lying across its centre (figs. 7 *b*, 8). In some cells at least this chromatin mass seems to divide, half passing to each end of the oval nucleus, and as this returns to its resting condition it is common to find a cap of chromatic material at each pole. The chromatin may finally disperse, taking the form of granules, and thus giving rise to a condition not differing greatly in appearance from the primary spermatocyte in which the chromatin has begun to appear. Possibly the division of the chromatin inside the nucleus, which occasionally seems to occur, is the persistent remnant of a true nuclear division, or it may perhaps be compared with the "intranuclear karyokinesis" described by Kostanecki† in parthenogenetic eggs of *Mactra*.

While the nucleus is returning to its resting condition, the apex of the pointed end of the cell, with the centrosome, becomes constricted off, apparently much in the same way as in the Bee. The amount of cytoplasm removed with the centrosome is very small, but it may sometimes be seen attached to the body of the cell by a narrow bridge containing spindle-fibres (fig. 7 *b*). In some cases the separation seems to be complete, and in follicles containing spermatocytes with a reconstituted nucleus small loose pieces of cytoplasm, sometimes with the centrosome visible at one end, are found scattered among the cells. In other cases these fragments seem to remain attached to the cell, but soon degenerate; in the prophase of the succeeding division they are perhaps represented by small knob-like excrescences sometimes found at the edge of the cell (figs. 8, 9, 10 *c*).

The cells with reconstituted nuclei may now be regarded as secondary spermatocytes, although no true nuclear division has occurred. In their resting condition, the duration of which it is not easy to determine, they do not differ greatly from the primary spermatocytes when the chromatin is beginning to appear; but they are generally more elongated, and very frequently the chromatin is closely packed round the nuclear membrane, especially at one pole, with fine strands radiating from this towards the more empty pole. At this stage they are much like the spermatids, in which the chromatin has a similar arrangement (*cf.* figs. 8 *c* and 17), but the nucleus is much larger, having the same size as that of the primary spermatocytes.

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* 'Proc. Amer. Acad. Arts and Sciences,' vol. 42, No. 5, fig. 8.

† 'Arch. Mikr. Anat.,' vol. 64, 1904, p. 1.

The chromatin next becomes grouped in the form of large elongate granules or small bands scattered under the nuclear membrane; their number is not easy to determine, but it approaches 20 (fig. 9). They then become combined, but whether by end-to-end pairing I cannot determine, into 10 very definite long bands having a more or less regular meridional arrangement under the membrane (fig. 10 *a*, *b*, *c*).

In one testis, in which a single follicle is much retarded, all the others containing well advanced spermatids, there are about 20 of these bands in each nucleus, and as they concentrate themselves to form chromosomes they appear to pair side by side to form 10 split chromosomes (Plate 1, figs. 18 *a*, *b*). It is possible that this lagging follicle is abnormal, for in all other cases where the bands can be counted their number is clearly about 10. The bands then become shorter, and arrange themselves across the nucleus in a fairly regular equatorial plate, in which 10 chromosomes radiate from a centre (figs. 11, 12).

The nucleus has now assumed the form of a wide spindle, stretching almost from end to end of the cell, but the membrane, though faint, appears still to persist. At each end of the nucleus a minute centrosome may be seen, and apparently within the nucleus is a system of spindle fibres extending from the centrosomes to the chromosomes, which lie across the centre. Commonly also at this stage a small deeply-stained dot may be seen outside the nuclear spindle, generally nearer one end. It is shown in figs. 13, 14, 15; where, as in figs. 13 *b*, 14 *a*, it appears within the spindle, it is in reality at a different level, and is always quite near the edge of the cell. The metaphase condition appears to last for some little time, for it is frequently found (fig. 13 *a*, *b*)

In the early anaphase the nuclear membrane has quite disappeared, and the chromosomes appear to split longitudinally, for in the equatorial plate they are arranged with their length across the spindle. The cell elongates somewhat, and the spindle extends completely from end to end. In early anaphase the chromosomes have the form of short rods converging towards the centrosomes (fig. 14 *a*, *b*); as they move apart they shorten, and as they aggregate round the centrosome they become still more concentrated. The cell meanwhile becomes constricted, a sheaf of spindle fibres extending from one daughter nucleus to the other, connecting the two halves (fig. 15 *a*, *b*). The two groups of chromosomes round themselves off into nuclei, having about half the size of the spermatocyte nuclei; the cells divide completely, and become a pair of spermatids (figs. 16, 17). In late anaphases the small stained body described above outside the nucleus may usually be seen included in one of the daughter cells; it is found outside the nucleus or spindle during the whole process, so cannot be regarded as any kind of accessory chromosome (figs. 12, 13, 14, 15).

It will be seen that the spermatogenesis in *Neuroterus* presents the same remarkable features as have been described by Meves* and Mark and Copeland† in the Bee and Wasp. In each we find the first spermatocyte division suppressed, being represented by the extrusion of a centrosome with a small quantity of cytoplasm.

Neuroterus resembles the Wasp in forming two nearly similar spermatids from each spermatocyte, but differs from both Bee and Wasp in having a definite resting stage between the abortive and true divisions.

The presence of the small stained body, persisting from the primary spermatocyte and passing into half the spermatids, has not been recorded in either Bee or Wasp.

Development of the Egg in the Ovary.

In rather young larvæ of the summer generation the ovaries consist of masses of cells not yet divided into egg-tubes, but they are beginning to arrange themselves into strands from which the egg-tubes will develop. No clear distinction is yet visible between the cells which will become eggs and those which will form the accessory cells between the eggs. The nuclei are mostly large, reticular, with one or two conspicuous nucleoli; others are stil larger, and have a mass of chromatic material aggregated in the form of dots and fine threads at one side of the nucleus. Nuclei like this may also be found in the eggs in the pupal egg-tubes. Mitotic figures are frequent; they resemble those found in the body-tissues, and clearly have the diploid number of chromosomes (Plate 1, fig. 19 *a*, *b*).

In young pupæ of the summer generation, the stages in the development of the ovarian egg can be followed easily, for the upper ends of the egg-tubes contain very early stages, and at the lower end well developed eggs are found. In the upper ends of the tubes all the cells are alike, but lower down differentiation takes place, and some cells enlarge greatly, become filled with yolk, and begin to develop into eggs surrounded by follicle-cells. Alternating with the developing eggs are masses of cells with large nuclei, each usually containing a double nucleolus; these cells are all alike, and do not differ much rom the primitive ova at the top of the egg-tube. The tube is thus moniliform, and the swellings contain alternately a developing egg and a mass of cells like primitive ova (fig. 20).

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* 'Arch. Mikr. Anat.,' vol. 70, 1907, p. 414; vol. 71, 1908, p. 571.

† 'Proc. Amer. Acad. Arts and Sciences,' vol. 42, 1906, p. 103; vol. 43, 1907, p. 71.

As the eggs develop they enlarge greatly by the deposition of yolk, and the nucleus appears as a relatively small vesicle, lying at one side. When ripe, the egg is prolonged at the front end into a hollow stalk with a small vesicle at the free end; the stalk is said to be held by the ovipositor as the egg is thrust into the hole bored in the leaf when the egg is being laid. The stalk thus partly blocks the opening, and in the spring eggs, which are generally somewhat compressed by the bud-scales, some of the yolk of the egg is frequently forced into the cavity of the stalk.

After one of the primitive ova has definitely begun to develop into an egg, as shown by the deposition of yolk, no division of the nucleus appears to take place until it is laid. In the follicle-cells which surround the eggs mitoses are not uncommon, and show the same number of chromosomes as in other somatic nuclei, viz., about 20.

In the females of the spring (agamic) generation, I have not observed the oogonia. In the pupæ the development does not differ materially from that of the summer generation. The egg-tubes are very similar, and the eggs when laid differ chiefly in being slightly larger. Since the pupæ develop very slowly, mitoses are more difficult to find, but the chromosome groups from an egg-follicle, represented in fig. 3, show that the somatic number is similar to that in the summer pupæ. Fig. 21 *a*, *b*, *c*, shows three figures of an egg-nucleus in which about 20 chromosomes appear to be visible, *a* and *b* being drawn from the same section at different levels, and *c* from the next section of the series.

Maturation and Fertilisation of the Egg.—Summer Generation.

The eggs laid by the flies of the summer generation in the early part of June are sunk in the tissue of the underside of young oak leaves, and the stalk of the egg commonly projects somewhat from the hole made by the ovipositor. These eggs are fertilised, and the large spermatazoon may almost always be found near the edge in sections of eggs preserved within about an hour and a half from the time of laying. The spermatozoon, after entering the egg, and while being converted into the male pronucleus, generally lies at the opposite side of the egg, or at least some distance removed, from the egg-nucleus, which during the same period is undergoing its maturation processes. During the first hour and a half after the egg is laid the nucleus is found near the edge; its position is somewhat variable, but it is commonly about midway between the ends of the egg (Plate 2, fig. 22).

In eggs preserved very shortly after being laid, the nucleus appears as a small darkly-stained body, either flattened against the edge of the egg or somewhat spindle shaped, with the longer axis of the spindle perpendicular to the edge. There is little doubt that the very narrow flattened nucleus is the earlier stage, but as both are found in eggs within half an hour of being laid, this is not perfectly certain. The nucleus now begins to enlarge, and instead of staining deeply and almost evenly throughout, chromatin bodies connected more or less conspicuously by a network appear within it (fig. 23). The succeeding stages are somewhat obscure, and are not unlike the maturation processes described by Henking* in the Gall-fly *Rhodites rosæ*. The nuclear membrane becomes faint and disappears, and the chromatin bodies segregate themselves to some extent into an inner and outer group, but whether by division of the individual chromosomes, or by a separation of chromosomes previously distinct, I cannot be sure (Plate 2, figs. 22, 24, 27, 30). The chromosomes of the inner group then separate themselves from those of the outer, and in so doing take the form of rods lying side by side or with their inner ends converging somewhat as in the anaphase of a typical mitosis (Plate 2, fig. 30). The outer part of the nucleus meanwhile has never become clearly separated into chromosomes, but appears as a group of chromatin bodies connected together by strands as in a reticular nucleus. Although Henking does not figure a stage exactly of this kind in *Rhodites*, yet he describes something similar. On pp. 149 and 150 he writes: "Die untere Tochterplatte lässt die Neunzahl unschwer erkennen, während in der äusseren Tochterplatte wiederum eine theilweise Verklebung eingetreten ist." In a nuclear division of this kind, in which there is never an equatorial plate, and in which the chromosomes of one half never become clearly separated, it is not easy to determine the chromosome number with certainty. But from a comparison of a large number of sections, especially when cut tangentially to the egg, it is fairly clear that the number of chromosomes in the inner group is about ten. In some sections not so many are visible, but that the number is really approximately ten there can be no doubt, *i.e.* the same number as in the spermatocyte divisions already described. In *Rhodites* Henking found nine.

The division just described does not lead to the production of two resting nuclei, but is succeeded immediately by further changes, which here also are obscure and hard to follow. Henking says that in *Rhodites* the chromosomes at each end of the first division-figure again divide, and thus give rise to four groups, of which the innermost forms a definite nucleus which sinks in as the female pronucleus, while the other three remain near the egg margin as polar bodies. Such a process would not be very different from what occurs in the saw-flies and other Hymenoptera, but Henking confesses that he has never actually seen this second polar mitosis. "Die Theilung muss ausserordentlich rasch verlaufen; denn . . . . habe ich hier immer gefunden, dass die Theilung der Chromosomen bereits vollendet war." Like Henking, I have had difficulty in observing this second polar division, although stages with three groups of chromosomes and a developing pronucleus are abundant. It is clear that the outer and inner groups left from the first polar division do not divide simultaneously, and although I have many sections of these stages I have no clear figure of the division of the inner group, *i.e.* of the separation of the female pronucleus from the chromosomes of the second polar body. For some time I doubted whether any such division occurred, but the presence of three groups of polar chromosomes is difficult to explain without it, and I have obtained a few sections which suggest that such a division is taking place. It clearly occurs before the division of the outer chromosome group, for while the latter is still a confused mass near the edge of the egg, the inner group often appears very much drawn out (Plate 2, figs. 26, 30) as if undergoing division, and in other sections (fig. 31) the division is seen completed. This stage seems to follow immediately on the first division, so that the division of the inner group of chromosomes is part of the same process as the original separation into inner and outer groups. In Plate 2, fig. 29, a different phase is represented, in which the inner group looks as if it were forming a compact mitotic figure, but this appearance is not usual.

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* 'Zeit. Wiss. Zoo.,' vol. 54, 1892, p. 147.

After the chromosomes which will give rise to the female pronucleus have sunk in to some extent, the outer group of chromosomes, lying near the edge of the egg, undergoes an irregular division (Plate 2, fig. 32), so giving rise to three groups altogether of polar chromosomes, but these are so confused and irregular that the number in each group is never ascertainable with certainty, and it appears as if some fusion often takes place between them (fig. 33).

There is one possibility which should be mentioned here, which is not entirely inconsistent with any of my sections. It is that the three groups of polar chromosomes are all derived by division of the outer group left by the first maturation division. I have no section which proves with certainty that the inner rod-like chromosomes described above are not converted direct into the female pronucleus, and that the three groups of polar chromosomes are not produced by a separation of the original outer group into two, followed by a second division of the outermost, and so yielding three groups. If this is the case, the innermost of the three groups must be formed by a sorting out of certain chromosomes from the original outer group, followed by a division of the remainder. It has been seen that there is no evidence for more than one maturation division in the spermatogenesis, but the existence of only one such division in the egg does not seem consistent with the presence of the diploid number of chromosomes in the oogonia.

The further fate of the polar chromosomes seems to vary somewhat in different eggs. In some, at the time of the conjugation of the male and female pronuclei, they appear as a single large group surrounded by a field clear of yolk granules, and with the individual chromosomes long and thread-like. Very frequently there are two groups, as if the two inner had amalgamated, and occasionally one finds three or four chromatin masses, in these cases usually with the chromatin closely balled together. In rather later stages, during the segmentation (about five to eight hours), the polar chromosomes seem to shrivel into small irregular masses and then to disappear completely.

It may perhaps be suggested that the apparent abnormality of the whole of the maturation processes of the egg is due to defective methods of preservation, but I think it unlikely for the following reasons. In the first place, at a rather later stage the conjugation of the pronuclei and segmentation divisions are well preserved; and secondly, Henking found very similar abnormalities in *Rhodites* in eggs preserved by hot water and by Flemming. It seems unlikely that three so different methods of preservation should all give the same results if the phenomena were not genuine. The division-figures in the cells of the leaf-tissue are also well fixed.

While the maturation of the egg-nucleus is in progress the spermatozoon is being converted into the male pronucleus. In eggs preserved within half an hour after being laid it commonly appears as a long narrow rod, straight or slightly curved, and often extending through two or even three sections. It contracts to a small, oval, compact nucleus, which stains very deeply, and then gradually swells, while chromatin bodies become visible within it. At this stage the chromatin sometimes suggests a coiled thread, but the staining is too dense to see whether this is so with certainty (Plate 2, fig. 32). As it increases in size, the male pronucleus sinks into the egg to meet the female pronucleus, which has now reached a similar condition, and when they meet in the centre of the egg they are both very large and vesicular (Plate 3, fig. 34).

The chromatin masses become definite chromosomes, the nuclear membranes disappear, and the two groups of chromosomes mingle and begin to form the first segmentation mitosis. Minute centrosomes are visible at the poles of the spindle, but I have not observed their origin. Sometimes at least the chromosomes in the equatorial plate of this first division are seen to be in two groups side by side, so that the complete mingling cannot take place till the first segmentation nuclei are reconstituted (fig. 35).

In the later segmentation mitoses the chromosomes are elongated, and appear to be about 18 to 20 in number; and in the nuclei just before mitosis a similar number of chromatin bands may be counted (figs. 36 to 38).

Maturation and Segmentation of the Egg of the Spring Generation.

My observations on the eggs of the spring generation are not yet complete, and cannot be finished until fresh material has been obtained. I give here a preliminary account of the results, some of which cannot be regarded as established with certainty, and leave the full description until the second part of this paper is published.

The eggs of the spring generation are parthenogenetic; they are laid in the developing buds of the oak in April, and as has been mentioned above, those laid by some females develop into males, those laid by others to females.

When the maturation divisions have been found they resemble the early stages of the maturation in the summer eggs, but I have never seen the division of the inner chromosome group, and am inclined to believe that it may sink in and become the egg-nucleus without further division, so that only one true maturation division takes place (Plate 3, figs. 39 to 43).

In other eggs I have been unable to find any trace of a maturation division, and in the later stages, during segmentation, no polar chromosomes are to be found in such eggs, although they are always to be found in the summer generation at the same stage. It seems probable, therefore, that there are two kinds of eggs, of which one undergoes a maturation division and the other does not, and this is confirmed by the study of the segmentation mitoses. In the eggs laid by the majority of spring females, 20 chromosomes are found in the segmentation mitoses (Plate 3, figs. 44 *a*, *b*; 45, 46 *a*, *b*, *c*), but in the eggs laid by one female the division-figures all contain 10 or about 10 chromosomes (figs. 47, 48 *a*, *b*, *c*), and in some of these eggs a double group of polar chromosomes is clearly recognisable at the edge of the egg in the place where the maturation mitoses have been found at an earlier stage. Most of my material was collected before I discovered that some individuals lay eggs which yield males, others which yield females, so that I have only one series of eggs laid by one female which show the reduced number in the segmentation divisions. But as far as they go, the results indicate that one kind of parthenogenetic female lays eggs which undergo no maturation, or at least no reduction, while the other kind lays eggs which undergo a maturation division, and in which the segmentation mitoses show the reduced number (10) of chromosomes. It has already been shown that in the male of the summer generation the spermatogonial divisions have 10 chromosomes, although the body-cells have 20, so that it is probable that the eggs which have a maturation division give rise to males, and it is possible that the 10 chromosomes seen in their segmentation mitoses are bivalent. In the body-cells these would then split into their univalent components, giving the 20 chromosomes observed, but in the developing germ-cells the haploid number of bivalents would be retained until the single spermatocyte division. In the summer females, on the other hand, the oogonia show 20 chromosomes, and these are probably produced by females which lay eggs that undergo no reduction.

The complete proof of these facts must be left until a fresh supply of material can be obtained in the spring.

Summary and Discussion.

The chief results described in the foregoing pages are the following:—

1. The Gall-fly, *Neuroterus lenticularis*, has two generations in the year, the flies appearing in April and June respectively. The spring generation consists exclusively of females, which differ considerably from the females of the summer brood. Their parthenogenetic eggs are laid in oak buds, and all the eggs laid by any one female develop into individuals of the same sex in June, *i.e.* some of the spring females are male-producing, others are female-producing. The summer generation thus consists of males and females; their eggs are fertilised, and are laid in the tissue of young oak leaves, and give rise to galls very different from those produced in the spring. The flies from these galls hatch in April and thus complete the cycle.

2. Mitoses in the body-tissues of young pupæ show about 20 chromosomes, both in the spring parthenogenetic females, and the males and females of the summer brood.

3. In the spermatogonia of young male larvæ, mitoses show 10 chromosomes. In the primary spermatocytes of young pupæ 10 chromosomes appear. An imperfect mitotic figure is developed, but the nuclear membrane does not disappear; and after the metaphase is reached the nucleus returns to a "resting" condition. During this process the cell develops an elongation at one end, at the tip of which is one of the centrosomes (or centrioles); as the nucleus re-forms, the centrosome and a small piece of cytoplasm are separated off, as happens in the Bee and Wasp. The process is thus much

like that found in the Bee, except that the nucleus returns to a "resting" condition.

4. The spermatocytes now develop about 20 chromatin masses which form themselves into 10 band-like chromosomes. These shorten, form an equatorial plate across the cell, a typical spindle is produced; and the chromosomes divide so that 10 travel into each daughter nucleus. Two spermatids are produced, which are similar except that in some cases at least one of them receives a small extranuclear body of unknown nature, which is absent from the other. Both spermatids develop into spermatozoa.

5. In primitive ova in the young female larvæ of the summer generation, mitoses like those in the body-cells are found, with apparently 20 chromosomes. After the deposition of yolk has begun, no further nuclear divisions occur in the egg.

6. The maturation divisions of the summer eggs are difficult to follow, but apparently two divisions occur, giving rise to four groups of chromosomes, of which the three outer represent the three polar nuclei, while the innermost group sinks in to form the female pronucleus. This group probably consists of 10 chromosomes, but they are so crowded that the number commonly appears rather less in sections.

7. The male pronucleus and the female pronucleus meet and form the first segmentation spindle, in which, as in the later segmentation divisions, about 20 chromosomes appear. The polar chromosomes disintegrate and disappear.

8. The primitive ova of the spring generation have not been observed. In an egg from the egg-tube of a young pupa 20 chromosomes were seen in the nucleus.

9. The maturation of the spring egg has not yet been sufficiently studied, but it appears that some eggs undergo at least one maturation division, others probably none. In eggs in which maturation has occurred segmentation mitoses show 10 chromosomes; all the eggs laid by one individual female in which the chromosomes could be counted were of this type, and it is suggested that these develop into males. In the eggs laid by other females, however, 20 chromosomes appear in the segmentation divisions; in these, polar chromosomes appear to be absent, and it is probable that there has been no maturation division, and that these eggs would develop into females.

Most of the above facts have been shortly discussed in the sections where they are described, and only their more important general bearings remain to be considered. Of these, perhaps the chief is the relation of the facts observed in *Neuroterus* to what is already known in other Hymenoptera, especially the Bee and other Aculeata. The life-history in the gall-flies is more complicated, involving an alternation of two different generations, but in some respects this simplifies our understanding of the facts. In the Bee and its relatives, fertilisation of the egg is facultative, and to all appearance, at least, the maturation of the egg is similar, whether fertilised or not. In the gall-flies the parthenogenetic eggs belong to a different generation, and since they give rise to both sexes, sex-determination in this generation, at least, does not depend on fertilisation. In the summer brood all eggs are fertilised and all give rise to females, so that here the gall-flies resemble the Bee. As in the Bee also, in the spermatogenesis there is only one maturation division of the nucleus, the number of chromosomes in the spermatogonial mitoses being the same as that entering the spermatids; in each case the first spermatocyte division is abortive, only a small portion of the cell being extruded. The Gall-fly differs from the Bee, but resembles the Wasp and Ant in producing two complete spermatids from each spermatocyte, while in the Bee one of the daughter nuclei degenerates without becoming a spermatozoon. It is tempting to speculate on the reason for this peculiarity in the Hive-bee, and a suggestion with regard to it will be made below after the parthenogenetic generation has been considered.

In the spring generation of *Neuroterus*, the eggs of which develop parthenogenetically, the evidence is unfortunately not yet complete. It has been seen that the eggs of some individuals give rise to females, those of others to males. The microscopical evidence, so far as it goes, suggests that in the eggs laid by some females there is possibly no maturation division, and in any case these eggs contain the diploid number of chromosomes; in eggs laid by other females a maturation division occurs, and the segmentation mitoses show the halved (haploid) number. It is suggested that the former type of egg develops into a female and the latter into a male, but since there is possibly only one maturation division in these, and since the body-cells of male pupæ contain the diploid number of chromosomes, it is possible that the chromosomes of the segmentation mitoses are bivalent. In the Bee also the body-cells of the male contain more chromosomes than the spermatogonia, in this case apparently four times as many (about 64 instead of 16).*

If it is the fact that parthenogenetic eggs of *Neuroterus* which have the diploid chromosome number in their segmentation mitoses develop into females, and those which undergo reduction become males, the sex-determination may be imagined as follows :—

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* Meves, 'Arch. Mikr. Anat.,' vol. 70, 1907, p. 420.

The egg which is to undergo maturation may be regarded as containing both male and female determinants; the female determinant is removed in the polar mitosis and the egg remains male. The eggs which will undergo no reduction may be considered as containing only a female determinant, and this causes the egg to develop into a female.

If, as suggested, the ovarian eggs of some parthenogenetic females contain both male and female determinants, while those of others contain a female determinant but not a male, the difference between the two kinds of eggs must be caused by a difference of constitution in the females which lay them. Both kinds of female are produced from fertilised eggs, and the difference in constitution between them may be due to the existence of two kinds of spermatozoa in the males of the previous generation. The existence of spermatozoa of two kinds is indicated by a number of facts which will be further discussed below, but here it will suffice to refer to the evidence in the Hymenoptera and other parthenogenetic insects. In *Neuroterus* itself I have mentioned above that half the spermatids receive an extranuclear body which is not found in the other half, and it is conceivable that this is connected with the difference under discussion. But the fact that in the Bee half the spermatid nuclei degenerate, while in other related Hymenoptera all develop into spermatozoa, suggests that two kinds of spermatozoa may exist. And a similar condition has been described by Morgan* in *Phylloxera*, and confirmed in a fuller paper by von Baehr† in *Aphis*. They find that there is an odd number of chromosomes in the somatic nuclei of the male, and at the first division of the spermatocytes one chromosome goes over undivided to one end of the spindle, so that one daughter nucleus receives a "heterochromosome," while the other does not. In the second division the heterochromosome divides equally, but only those secondary spermatocytes which contain it develop further, the others degenerating. Thus, as in the Bee, half the daughter-cells of the primary spermatocytes atrophy, and since all fertilised eggs become females, Morgan supposes that the heterochromosome, which is contained in the functional spermatids, bears the female determinant. A different explanation of the facts will be offered below, but the facts themselves clearly indicate the existence of two kinds of spermatids, one of which, as in *Neuroterus*, contains a body lacking in the other. We may suppose, then, that in *Neuroterus*, one kind of spermatozoon, fertilising an egg of the summer generation, gives rise to female-producing (thelytokous) female, the other kind to a male-producing (arrhenotokous) female. In the Bee, as far as is known, all queens produce similar eggs, and this would be accounted for by the degeneration of one kind of spermatozoa.

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* 'Proc. Soc. Exper. Biol. and Med.,' 1908, vol. 5, p. 56. (Full paper, 'Journ. Exp. Zoo.,' vol. 7, 1909, p. 239.)

† 'Zool. Anzeiger,' vol. 33, 1908, No. 15.

The sex-determination in the Gall-fly would then take place as follows:—The spermatocytes of the male would bear a determinant for maleness, which may be represented by the symbol ♂. At the nuclear division this passes into one daughter-cell, its fellow being without it and thus containing no sex-determinant, a condition which may be represented by the symbol ⊙. The fertilisable eggs after maturation are supposed all to bear one determinant for femaleness, represented by the symbol ♀. The zygotes all develop into parthenogenetic females, but since half of them were fertilised by ♂-bearing spermatozoa, and half by spermatozoa with no sex-determinant (⊙), the former will have the constitution ♀ ♂, the latter ♀ ⊙. The females with constitution ♀ ♂ lay eggs which undergo maturation; the ♀ determinant is expelled, and the egg left with the ♂ determinant becomes a male. The females with constitution ♀ ⊙ lay eggs which undergo no reduction, but containing the ♀ determinant develop into females. The ♀ determinant is transmitted to the eggs of the summer generation, and since only one sex-determinant is present in the egg, it remains in the pronucleus when the polar mitoses take place, with the result that all eggs of the summer generation are ♀-bearing, as is assumed above.

It now remains to be seen whether this scheme is consistent with what is known of sex-determination, first in ordinary cases where all eggs are fertilised and secondly in the Bee and other aculeate Hymenoptera. The ordinary bisexual cases will be considered first.

As the result of my work on heredity and sex-determination in the moth, *Abraxas grossulariata*,* and of other similar cases, Bateson† suggested the hypothesis that the female is heterozygous in respect of sex, containing male and female determinants, the male homozygous containing only male. Eggs would thus be produced in equal numbers bearing maleness or femaleness, but all spermatozoa would bear the male determinant. This hypothesis would completely explain the cases which led to its formulation, but it is not completely consistent with the existence in many insects of two kinds of spermatozoa, one containing a heterochromosome and one without it. More recently, the evidence from some forms of sex-limited inheritance, such as colour-blindness‡ and congenital nystagmus,§ have made it clear that there are not only two kinds of eggs differing in their sex-determinants, but also two kinds of spermatozoa. In colour-blindness, for example, the affection is dominant in the male and recessive in the female; so that heterozygous males exhibit it, heterozygous females do not; but affected men do not transmit it to their sons. The sons of affected males are normal, but the daughters of affected males transmit the disease to some of their sons and, through some of the daughters, to their grandsons. It is clear, therefore, that among the spermatozoa of an affected male, those only bear the determiner for the disease which will give rise to female zygotes; but among the ova of a female heterozygous for the colour-blind condition, the colour-blind factor is borne indiscriminately by about half the ova, whether male-producing or female-producing.

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* 'Evol. Committee Roy. Soc., Report IV,' 1908, p. 53.

† 'Science,' 1908, N.S., vol. 27, p. 785.

‡ Bateson, "Mendel's Principles of Heredity," 'Camb. Univ. Press,' 1909, 1st impression, p. 230, inserted slip; 2nd impression, 1909, p. 195, note.

§ See Lloyd Owen, 'Ophthalmic Review,' vol. 1, 1882, p. 239.

These different facts all fall into line if we assume that the female contains both female and male sex-determinants, and produces equal numbers of ova bearing each; while the male contains no female determinant, but produces two kinds of spermatozoa, one bearing the male determinant, the other being without any determinant for either sex. Using the same symbols as before, viz., ♀ and ♂ for the female and male sex-determinants respectively, and the symbol ⊙ for absence of sex-determinant in a gamete, females would then have the constitution ♀ ♂, and would produce ♀ and ♂ eggs in equal numbers, males would be ♂ ⊙, and produce spermatozoa ♂ and ⊙ in equal numbers. In the insects with heterochromosomes, in the male the heterochromosome is regarded as bearing ♂, its absence being represented by ⊙; in the female the two heterochromosomes bear ♀ and ♂ respectively. If we regard the sex-determinants as equivalent to Mendelian allelomorphs, they must be considered as two pairs, each determinant being allelomorphic with its absence, *i.e.* ♀ with ⊙, and ♂ with ⊙, but ♀ and ♂ are spuriously allelomorphic with each other, so that when they coexist in the same zygote they cannot both enter the same gamete; just as in the case of *Abraxas*, the ♀ determinant and the *grossulariata* determinant exhibit spurious allelomorphism, and are never found in the same gamete. We shall then have females producing equal numbers of ♀ eggs and ♂ eggs, males producing equal numbers of ♂ and ⊙ spermatozoa, and it must further be assumed that ♀ eggs are fertilised by ♂ spermatozoa, giving females (♀ ♂), ♂ eggs by ⊙ spermatozoa, giving males (♂ ⊙).

This scheme is consistent with the facts in three categories of cases which have hitherto seemed irreconcilable, viz., *Abraxas* and those cases which resemble it; the insects with heterochromosomes, and the cases of sex-limited inheritance of which colour-blindness is the type. In *Abraxas* there is spurious allelomorphism between the ♀ determinant and the *grossulariata* factor, so that ♀ eggs are without that character; ♂ eggs have it, but in the male, since the ♀ determinant is absent, there is no such spurious allelomorphism, and the *grossulariata* factor is indiscriminately distributed among the spermatozoa. In insects with heterochromosomes these may be regarded as bearing the ♂ and ♀ factors as suggested above. In colour-blindness, the factor for the disease can only be borne by a gamete which contains a sex-determinant, but the latter may be either ♂ or ♀; in the male, the factor is borne by the ♂-bearing spermatozoa, but not by those which have no sex-determinant (⊙); since the latter fertilise ♂-bearing ova and the former ♀-bearing, an affected male transmits the factor for the disease only to his daughters. But in the heterozygous female the colour-blind factor may be associated with either ♂ or ♀ eggs, and thus some of her children of each sex receive it.

The suggestion may here be made in passing that this hypothesis of sex-transmission may explain the cases not rarely met with, especially in the offspring of hybrids, in which all or nearly all the offspring are of one sex. If a ♀ egg were occasionally fertilised by a ⊙ spermatozoon, the resulting individual would be a female containing no male determinant, and either all its eggs would be ♀-bearing, or if any contained no sex-determinant they might be sterile or unfertilised. In crosses which give only male offspring (as in the case of the moths, *Tephrosia crepuscularia* and *T. bistortata* described by Tutt*), the ♀-bearing eggs may fail to attract the spermatozoa of the other species, and so only male offspring would result.

After this digression, we must now return to the case of the Bee, Wasp, and other parthenogenetic cases. In a previous paper† I have made the suggestion (which I find has also been made by Morgan) that the presence or absence of a spermatozoon in the egg might cause the maturation to take place differently. It is possible that when a spermatozoon is present the egg of the Bee undergoes normal "reduction" divisions, halving the chromosomes both quantitatively and qualitatively, and removing the ♂ determinant. But when the egg develops unfertilised the polar divisions may both be equational, leaving the haploid (reduced) number of bivalent chromosomes in the egg, and in this case expelling the ♀ determinant. This would be comparable with the supposed male eggs in the spring brood of *Neuroterus*, and in each case in the germ-cells of the male the reduced number would remain throughout, but in the body-cells the bivalent chromosomes would separate into their constituents, so giving the same number in male and female body-cells, as is observed. In the male Bee a single ♂ determinant would be present in the spermatocytes: this would pass into one spermatid, the other being left with none (⊙). The latter spermatids are the "male polar bodies" which degenerate. All the spermatozoa thus contain the ♂ factor, their presence in the egg causes the ♀ factor to remain in the polar divisions and the fertilised egg is thus ♀ ♂ and becomes a female.

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* Tutt, 'Trans. Ent. Soc.,' 1898, Part I, p. 17. Quoted by Castle, "Heredity of Sex," 'Bull. Mus. Comp. Zoo. Harvard,' vol. 40, 1903, p. 206.

† "On the Maturation of the Egg in the Teuthredinidæ," 'Q.J.M.S.,' vol. 49, 1906, p. 586.

This assumption that the presence of a spermatozoon in the egg causes a difference in the maturation process, and leads to the removal of the male instead of the female sex-determinant seems improbable at first sight, but there are considerations which reduce its improbability on further examination. If both eggs and spermatozoa are commonly of two kinds, the hypothesis of selective fertilisation cannot be avoided, and if there is some definite attraction between a ♂-bearing and ♀-bearing nucleus, the presence of a ♂-bearing spermatozoon (or male pronucleus) in the egg may attract the ♀ determinant to the inner end of the maturation spindle, although in an egg with no spermatozoon it would be expelled. Clearly some such attractive force exists, the nature of which is at present unknown, for otherwise in the saw-flies the male pronucleus might conjugate equally often with one of the polar nuclei, which to all appearance exactly resemble the egg-nucleus, instead of with the egg-nucleus itself. If it is assumed that, in cases where the egg matures before the entrance of the spermatozoon, the sex-determinant remaining in the egg attracts only one of the two kinds of spermatozoa, it does not seem a very improbable extension of the hypothesis that the presence of one kind of spermatozoon in the unmatured egg may cause the expulsion of the corresponding sex-determinant at the maturation division.

In Wasps and Ants* the spermatogenesis takes place much as in the Bee, except that two apparently similar spermatids are formed by the spermatocyte division, each of which becomes a spermatozoon. Their spermatogenesis thus closely resembles that of *Neuroterus*, and the most probable assumption seems to be that two kinds of spermatozoa are formed, ♂ and ⊙ respectively, but that only the ♂-bearing are functional. They would thus be exactly comparable with the Bee, except that the reduction of the useless spermatozoa is not carried so far. Other possibilities are of course not excluded, *e.g.*, that the ♂ determinant divides and passes into both spermatids, or that, as in *Neuroterus*, functional ♂ and ⊙ spermatozoa occur, and therefore that among the offspring of the female which founds the nest there may be two kinds of female individuals produced, one of which would contain no ♂ determinant. In ants and wasps the workers are not sharply separated from the queens as they are in the Bee, and these purely female individuals might develop into workers which would not lay eggs. It is generally considered that when worker ants lay eggs, these always yield males (as in the Wasp and Bee), but Reichenbach* describes the case of a nest in which the workers produced females except at the season when males normally occur, and then males were produced. If some of the workers were of the ♀ ⊙ constitution, others ♀ ♂, this might thus be explained. The case clearly requires further investigation.

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* Meves and Duesberg, 'Arch. Mikr. Anat.,' vol. 71, 1908, p. 572.

The hypothesis of sex-determination outlined above is doubtless highly speculative, but it has the advantage that it brings into line several sets of facts which have hitherto seemed irreconcilable, viz., the results obtained by breeding such cases as *Abraxas*, and also such cases as colour blindness, the cytological observations of Wilson and others on heterochromosomes, and the peculiar behaviour of the Bee and other Hymenoptera which have facultative parthenogenesis.

The fact that in *Neuroterus* the parthenogenetic generation is separated from the bisexual one, and that some parthenogenetic females give rise only to males, others only to females, supplies the clue, for it is clear that some difference must exist in the constitution of the spring-brood females to account for the difference in sex in the offspring of different individuals. That this factor is introduced by the male parent seems probable from the known fact that in so many cases spermatozoa of two kinds are produced.

In the Aphides a variety of conditions occurs in different cases, but here also the suggested explanation holds good. In all species fertilised eggs yield females, and these produce a varying number of parthogenetic generations. All these must be regarded as of constitution ♀ ♂. In some, the later parthenogenetic females give rise to both sexual females and males from the same individual;† in these it may be assumed that in the eggs which develop into sexual females the ♂ determinant is removed; from those which yield males the ♀ determinant. In other cases the parthenogenetic females give rise to "sexuparæ," some of which produce parthenogenetically only males, others sexual females. In these, the female-producing sexuparæ may have lost the ♂ determinant, while the male-producing sexuparæ contain both ♂ and ♀, but the ♀ determinant is removed with the polar body of the egg which yields a male. Morgan and von Baehr have shown in a species of *Phylloxera* and in *Aphis saliceti* that half the secondary spermatocytes degenerate, as do half the spermatids in the Bee, and in this case they have shown that those which degenerate lack the heterochromosome, *i.e.* those which we have represented by ⊙. The spermatozoa are thus all ♂-bearing, but since the sexual female lacks the ♂ element, all the eggs are ♀, and the resulting offspring (zygotes) are females with constitution ♀♂. Morgan assumes that the spermatozoon determines the sex, but this involves the belief that the male Aphid contains a ♀ determinant, and since we know that the parthenogenetic female contains the ♂ factor (since males are produced from it), this would involve the complex assumption of alternative sex-dominance. If my hypothesis is correct, it could be tested in Aphis by observing whether, in the single polar division by which a male egg is produced, one complete heterochromosome is extruded, thus leaving only one heterochromosome in the egg instead of the two which are characteristic of the parthenogenetic females.

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* 'Biol. Centralblatt,' vol. 22, p. 461.

† N. M. Stevens, 'Carnegie Inst. Public., Washington,' No. 51, 1906; also von Baehr, *loc. cit.*

In this discussion I have not attempted to deal with the case of the saw-flies (Tenthredinidæ), in which facultative parthenogenesis occurs. In former papers* I have discussed this question, but subsequent observations have led me to believe that the work requires extension and revision, and since this is still in progress, I prefer to leave any discussion until the results are clear.

A final word should be given to the recent hypothesis of Wilson† and Castle,‡ that the sex-determinants are not ♂- and ♀-bearing respectively, but that only one kind of determinant exists, and that the female contains one more such determinant than the male. Castle supposes that in some species the female contains two such determinants, the male one; in other species, the female one and the male none. The existence of two kinds of parthenogenetic females in *Neuroterus* cannot be explained on either assumption, but it is conceivable that both kinds of parthenogenetic female contain one sex-factor and the male none. The difference between the arrhenotokous and thelytokous female would then be that the eggs of the former contained a mechanism for expelling the sex-factor, while the thelytokous egg would retain it. The extranuclear body found in half the spermatids might be responsible for this difference. This, however, does not seem very probable. The hypothesis, also, cannot explain the almost invariable excess of affected males in cases of sex-limited inheritance, such as colour-blindness, which is accounted for if we assume that the factor for the disease is more often borne by ♂ than by ♀ ova. In general, the hypothesis does not seem to bring together such a wide range of phenomena as that suggested above.

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* 'Q.J.M.S.,' vol. 49, 1906, p. 561 and vol. 51, 1907, p. 101.

† 'Science,' January, 1909, vol. 29, p. 53.

‡ 'Science,' March, 1909, vol. 29, p. 395. See also Morgan, 'Journ. Exp. Zoo., Sept., 1909, p. 332.

In conclusion, I wish to acknowledge the assistance received in the later part of my work from a grant from the "Endowment of Research Fund" of the Birmingham Natural History and Philosophical Society, and especially to record my indebtedness to Prof. Bateson, who has read the MS. of the paper and given valuable help in criticisms and suggestions.

EXPLANATION OF PLATES.

The figures, with the exception of Nos. 20 and 22, were drawn with Zeiss apochromatic objective, 3 mm. (apert. 1·40), and Zeiss ocular 12, giving a magnification of about 1600 diameters. When the magnification is less, it is mentioned in the description of the figure. The outlines of the larger figures were drawn with camera lucida, but the chromosome groups are drawn free-hand.

Plate 1.

Fig. 1.—Chromosome group from anaphase of mitosis in developing wing of old larva, male.

Fig. 2.—Two similar groups from female pupa, summer generation.

Fig. 3.—Two similar groups (the two ends of one mitotic figure) from a pupa of the spring generation.

Fig. 4 *a*, *b*, *c*.—Three anaphases, two in face and one side-view, of spermatogonial mitoses, half-grown larva.

Fig. 5 *a*, *b*.—Two primary spermatocytes, after the growth period. Male pupa.

Fig. 6.—Prophase of first spermatocyte mitosis. Chromosomes appear as about 10 irregular bands.

Fig. 7 *a*.—Early stage of first (abortive) spermatocyte division. A centriole is seen at each end of the cell, and a small dot on the upper edge of the nucleus.

Fig. 7 *b*.—Later stage: the nucleus is re-forming, and the cytoplasmic bud with the centriole is being separated.

Fig. 7 *c*.—Rather earlier stage: pear-shaped nucleus and finger-like process with centriole at its tip.

Fig. 8 *a*, *b*, *c*.—Secondary spermatocytes, three stages of re-formation of the nucleus. In 8 *b* and *c* the cytoplasmic bud at one end, and the stained dot near the nucleus, are visible.

Fig. 9.—Early prophase of second mitosis. More than 10 chromatin bodies are visible, although part of the nucleus is not included in the section. At this stage the chromatin bodies are not always so definite as in this case.

Fig. 10 *a*, *b*, *c*.—Three prophases of second spermatocyte mitosis, showing 10 band-like chromosomes. In 10 *a* not all the chromosomes are represented; in 10 *c* the little knob on the lower side of the cell perhaps represents the "cytoplasmic bud."

Figs. 11 and 12.—Equatorial plates of second mitosis; in 12 the stained dot is seen outside the circle of chromosomes.

Fig. 13 *a*, *b*.—Two metaphases in side view.

Fig. 14 *a*, *b*.—Anaphases in side view. In each the stained dot is seen near the lower end of the spindle.

Fig. 15 *a*, *b*.—Telophases: stained dot seen included in one daughter-cell.

Fig. 16.—Two telophases seen in pole-view.

Fig. 17.—Two early spermatids.

Fig. 18 *a*, *b*.—Two prophases from a probably abnormal follicle. In 18 *a* the double number of chromosomes is seen, in 18 *b* they appear to be pairing longitudinally. In 18 *b* not all the chromosomes are shown.

Fig. 19 *a*, *b*.—Anaphases from mitoses in ovary of half-grown female larva, summer generation. *a*, polar view; *b*, side view.

Fig. 20.—Part of egg tube from pupa of summer generation, showing alternation of developing egg on right, with undifferentiated primitive ova on left. (× about 650.)

PLATE 2.

Fig. 21 *a*, *b*, *c*.—Nucleus of developing egg of spring generation. *a* and *b* are optical sections of the nucleus at different levels, *c* part of the same nucleus in the next section of the series. In *b* the chromosomes shown in outline are ends of those seen in the same positions in *a*.

Figs. 22 to 33 represent the maturation of the summer egg.

Fig. 22.—Outline of section of egg showing stalk and polar mitosis. (× about 650.)

Fig. 23.—Nucleus at edge preparing for maturation division.

Fig. 24 *a*, *b*.—Two successive sections of early stage of division.

Fig. 25 *a*, *b*.—Polar views of first maturation division. *a* represents the inner, *b* the outer chromosome group. Drawn from different levels in the same section. Fig. 27 represents nearly the same stage in side view.

Fig. 26 *a*, *b*, *c*.—Later stage of maturation. *a* and *b* are drawn from different levels in the same section, *c* from the next section of the series. *a* represents the innermost group, and some of the chromosomes in *b* are apparently continuous with those in *a*. *c* represents the outermost group.

Fig. 27.—Early stage of the first maturation division, side view.

Fig. 28 *a*, *b*, *c*, *d*.—*a* and *b* are the two groups of polar chromosomes drawn from the same section at different levels. *c* represents the spermatozoon becoming the male pronucleus in the next section; *d*, the insinking chromosomes forming the female pronucleus cut twice in the succeeding two sections.

Fig. 29.—Early stage of second maturation division, unusual condition.

Fig. 30 *a*, *b*.—Close of first and beginning of second maturation divisions; two successive sections of series. *a* shows outer group, *b* part of outer and elongated inner group.

Fig. 31.—End of second division of inner group. Chromosomes which form female pronucleus closely packed, below; inner polar chromosomes and outer group preparing to divide, above. Female pronucleus in next section to polar chromosomes. (× about 800.)

Fig. 32.—Close of second maturation division. Chromosomes of female pronucleus sinking in, outer group completing its division. *sp.* represents the spermatozoon becoming the male pronucleus, from the next section. (× 800.)

Fig. 33.—Female pronucleus (*pr. n.*) and three groups of polar chromosomes.

PLATE 3.

Fig. 34.—Meeting of male and female pronuclei.

Fig. 35.—Conjugation of pronuclei and first segmentation spindle. *a* and *b* mark the chromosome groups derived from the two pronuclei. In the section they lie at different levels, so that they are actually placed across the axis of the spindle.

Fig. 36 *a*, *b*.—Two polar views of telophases of segmentation mitoses, showing about 20 chromatin masses. Side view of same stage in fig. 38.

FIGS. 37 and 38.—Two stages of segmentation mitoses.

FIGS. 39 to 48 represent eggs of the spring generation.

FIG. 39 *a*, *b*.—Two successive sections of chromosome group at edge of egg before maturation division.

FIG. 40.—Early stage of maturation division.

FIG. 41.—Late stage of maturation division.

FIG. 42.—Completion of maturation division. Chromosomes of egg-nucleus sinking in; polar chromosomes in two groups.

FIG. 43 *a*, *b*, *c*.—Polar chromosomes and egg-nucleus. *a* and *b*, successive sections of polar chromosomes; *c*, egg nucleus several sections removed. (× about 800.)

FIG. 44 *a*, *b*.—Two equatorial plates of segmentation mitoses, showing about 20 chromosomes.

FIG. 45.—Prophase of segmentation mitosis, showing numerous long coiled chromosomes.

FIG. 46 *a*, *b*, *c*.—Metaphase (*a*), early (*b*), and late anaphases (*c*) of normal segmentation mitoses with diploid number.

FIG. 47.—Equatorial plate in face of segmentation mitosis with haploid number.

FIG. 48 *a*, *b*, *c*.—Metaphase (*a*), anaphase (*b*) in side view, and anaphase in pole view (*c*) of segmentation mitoses with haploid number.

Preliminary Note upon the Cell Lamination of the Cerebral Cortex of Echidna, with an Enumeration of the Fibres in the Cranial Nerves.

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(Communicated by Dr. F. W. Mott, F.R.S. Received September 30,—Read December 9, 1909.)

[PLATES 4 AND 5.]

Material.—The following notes are based on the study of the brain of an Echidna which died in the gardens of the Zoological Society in London.

Dr. F. W. Mott, F.R.S., kindly placed the brain in my hands with the suggestion that I should examine the cell lamination of the cortex and should estimate the numbers of fibres in the cranial nerves. For this and for his advice and help during the investigation I wish here to express my gratitude.

The right hemisphere was cut transversely into a series of sections 10 μ in thickness, from which sections were taken at intervals of about $\frac{1}{2}$ mm., stained with polychrome blue, and mounted. It may, perhaps, be mentioned that the sections were somewhat erratic in their behaviour towards the stain, and required a prolonged immersion in it. The preservation of the cells was not sufficiently good to justify any minute histological description, and therefore none such has been attempted, nor has the large variety of cell outlines been described. A great deal of this apparent diversity must be due to the cutting of similar cells in slightly different planes, and to the many aspects which cells of the same type must present to the observer according as they are placed in this position or that. Moreover, it was felt that the drawings (figs. 3, 4, 5, 6) in which the cells are represented in silhouette display both their shape and their arrangement more clearly and more briefly than could any verbal description.

Owing to the general shape of the hemisphere and, more particularly in its posterior part, to the course of the fissures, transverse sections in the neighbourhood of the two extremities are confusing on account of the obliquity with which the cortex is cut. In order to obviate this difficulty sections were cut from the left hemisphere in such a way that they met the fissures as much as possible at right angles. In spite of this it has not been found possible to give any adequate presentment of the cortex at the anterior and posterior extremities of the brain, but it is hoped to obtain fresh material with which the gaps in the present paper may be filled up.

Ziehen* gives descriptions of certain types of cortex to be found in the brain of Echidna but no figures either of the cell lamination or to explain in what part of the hemisphere the structures he describes may be found. As I could not from his verbal descriptions get any clear notion on the latter point, I have omitted to compare his descriptions with my own.

Surface Anatomy of the Brain (*vide* fig. 1).

Before beginning a study of the transverse sections, in order that these may be understood, it may be as well to say something of the arrangement of the fissures. The latter have been described by G. Elliot Smith† and by Ziehen.‡ The descriptions agree in all essentials, but whereas Elliot Smith designates the different fissures by letters of the Greek alphabet, Ziehen provides them with long Latin names indicating their positions on the brain.

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* "Das Central-Nerven-System der Monotremen und Marsupialer," 2. Theil, Microscopische Anatomie; 'Jenaische Denkschr.,' VI, 2. Theil; Semon, 'Zoolog. Forschungsreisen,' III, 2. Theil, p. 848.

† Museum of the Royal College of Surgeons of England, Catalogue of Physiological Series, vol. 2, second edition, 1902.

‡ "Das Central-Nerven-System der Monotremen und Marsupialer," Semon, 'Zoolog. Forschungsreisen,' III, p. 8; 'Jenaische Denkschriften,' VI.

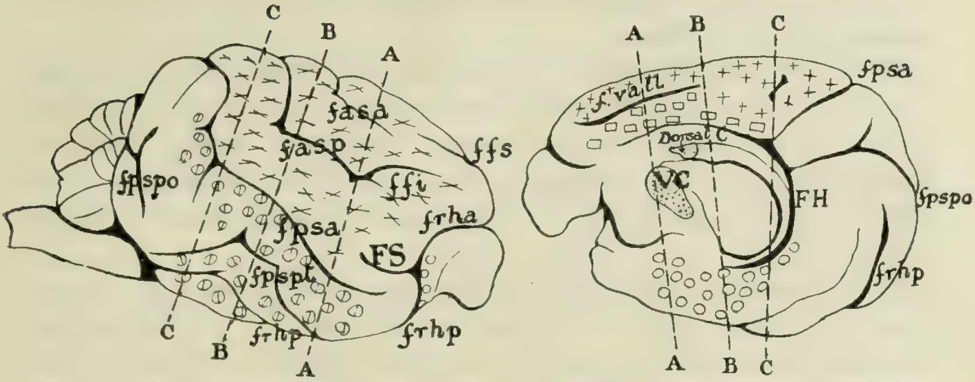

Fig. 1.—A. Fig. 1.—B.

Fig. 1.—Diagram of right hemisphere of Echidna. × 2½. A, Lateral aspect; B, Median aspect.

Fissures.

FS	Fissura Sylvii.
frha	„ rhinica posterior.
frhp	„ rhinica anterior.
fpsa	„ postsylvia anterior.
fpspo	„ postsylvia posterior occipitalis.
fpspt	„ postsylvia posterior temporalis.
fasp	„ antesylvia posterior.

fasa	Fissura antesylvia anterior.
ffs	„ frontomarginalis superior.
ffi	„ frontomarginalis inferior.
FH	„ hippocampi.
Fvall	„ vallaris.
VC	Anterior (ventral) commissure.
Dorsal C.	Dorsal commissure.

A – – – *A* shows plane of Section *A*.
B – – – *B* „ „ *B.*
C – – – *C* „ „ *C.*

Types of Cortex.

□ □ □	Type I.
+ + + +	Type II.
× × × ×	Type III.
⌽ ⌽ ⌽ ⌽	Type IV.
○ ○ ○ ○	Type V.

Ziehen's names, though clumsy, have the advantage that they remind one of the position of the fissure referred to, and it is for this reason that they are adopted here.

He takes as his starting point the—

Fissura Sylvii (*FS*).—This is generally about 5 mm. in length, and runs almost horizontally backwards. From its anterior end the fissura rhinica anterior runs forwards and the fissura rhinica posterior runs backwards.

Fissura rhinica anterior (*frha*) runs horizontally forwards and reaches the mesial surface. Its incision of the mantle edge delimits the olfactory lobe or bulb. The greatest depth of the fissure is 7 or 8 mm., but the incision referred to is 9 mm. deep.

Fissura rhinica posterior (*frhp*) runs at first basalwards, then turns horizontally backwards, and describes finally a flat curve with its concavity directed downwards. Its posterior segment, sometimes separated from the main division, cuts the posterior mesial border of the hemisphere. The depth in the anterior division is about 7 mm., but gets considerably less posteriorly.

Fissura postsylvia anterior (*fpsa*) [Elliot Smith, α] approaches the sylvian fissure at its lower end in a characteristic manner; it runs upwards and backwards and cuts the mesial border of the hemisphere 25 mm. from the frontal pole. It may be straight or wavy, but there is always an angular bend in the middle third from which a posteriorly directed side branch is given off. The depth is between 2½ and 4½ mm.

Fissura postsylvia posterior (*fpsp*) lies 6 mm. behind *fpsa*; it cuts the border of the hemisphere behind the occipital pole, *i.e.* behind the point where the border runs laterally outwards. The fissure runs continuously downwards and forwards as far as the angle of *fpsa*, but in front of this its direction is continued by an anterior segment. The main fissure is called f. postsylvia posterior occipitalis (*fpspo*) [Elliot Smith, ζ], and is 2½ mm. deep. The anterior segment is called f. postsylvia posterior temporalis (*fpspt*) [Elliot Smith, η], and is 1½ mm. deep. Occasionally another fissure, f. postsylvia postrema, is present behind and parallel to *fpsp*.

Fissura antesylvia posterior (*fasp*) [Elliot Smith, β] is the most constant of the fissures in the anterior part of the brain; it lies parallel to *fpsa*, 2 to 6 mm. in front of it. When well developed its lower end lies over the sylvian fissure. In this region it often turns sagitally forwards and may be forked. Its depth may reach 3 mm. Between it and *fpsa* there may be another fissure.

Fissura antesylvia anterior (*fasa*) [Elliot Smith, γ and δ) lies parallel to and in front of *fasp*. It is sometimes as well developed as the latter, and cuts deeply into the mesial border of the hemisphere. More often it is weakly developed, failing to reach the border and sometimes falling into two pieces. Its lower end may be forked, depth to 3 mm.

Fissuræ frontomarginales superior and inferior (*ffs* and *ffi*) [Elliot Smith, ε] lie in front of *fasa*; they are very variable.

On the median surface of the brain may be found the *Fissura hippocampi* (*FH*) which follows the characteristic course of the hippocampus, starting in the upper anterior part of the brain, running backwards and then curving downwards and finally forwards, to end somewhere below its starting point.

Fissura vallaris (*f vall*) runs parallel to the border of the hemisphere in the anterior part of the brain. Its length is about 10 mm. Its posterior end lies above the anterior commissure, its anterior end 5 to 6 mm. from the frontal pole.

Radial Fissures.—Three or four may be present, of which the anterior may be a median prolongation of *fasa.* The second is sometimes independent, sometimes a prolongation of *fasp.* The third is generally very sharply defined, and is always in continuity with *fpsa*; it may have the appearance of being connected with *FH.* The fourth is often very weak and is usually independent.

Elliot Smith says that the fissure ψ is the deepest and most constant of the radiating sulci in the mesial surface of the brain. In most brains it crosses the dorsal edge and joins β (*fasp*) in the dorsal surface.

Description of Five Distinct Types of Cell Lamination, with Notes as to Their Distribution.

The sections A, B, and C, diagrammatically represented in fig. 2, are intended to illustrate the distribution of the various types of cortex in the middle region of the hemisphere. The position of these sections which are cut transversely is shown in fig. 1. This figure also shows the distribution of the types of cortex on the surface of the brain. From it and from the foregoing descriptions the manner in which the various fissures are cut may be understood.

Type I. □ □.

This cortex measures only 1 mm. in depth, and is not very highly differentiated. The molecular layer occupies rather less than 0·1 mm. It is succeeded by a layer of broad and irregular rectangular or pyramidal cells, which stretch downwards to a depth of 0·5 mm. While in the upper region of this layer the cells are small (15 to 20 μ in length), they become considerably larger (20 to 27 μ) in the neighbourhood of its lower boundary, and are there more regular in shape and arrangement. The rest of the cortex is made up of small polymorphic cells. A few granules are found in the polymorphic layer and in the lower part of the pyramidal layer.

Type I may be found in the median wall of the hemisphere, lying between the *Fissura vallaris* (*F vall*) and the *Fissura hippocampi* (*FH*). It extends backwards some way behind the posterior end of the former. It may be seen in practically the same position in sections A, B, and C. In section A the Hippocampal fissure is quite shallow, but posterior to this it gets much deeper, and as it deepens the cortex originally lying above it becomes invaginated so as to clothe its upper wall.

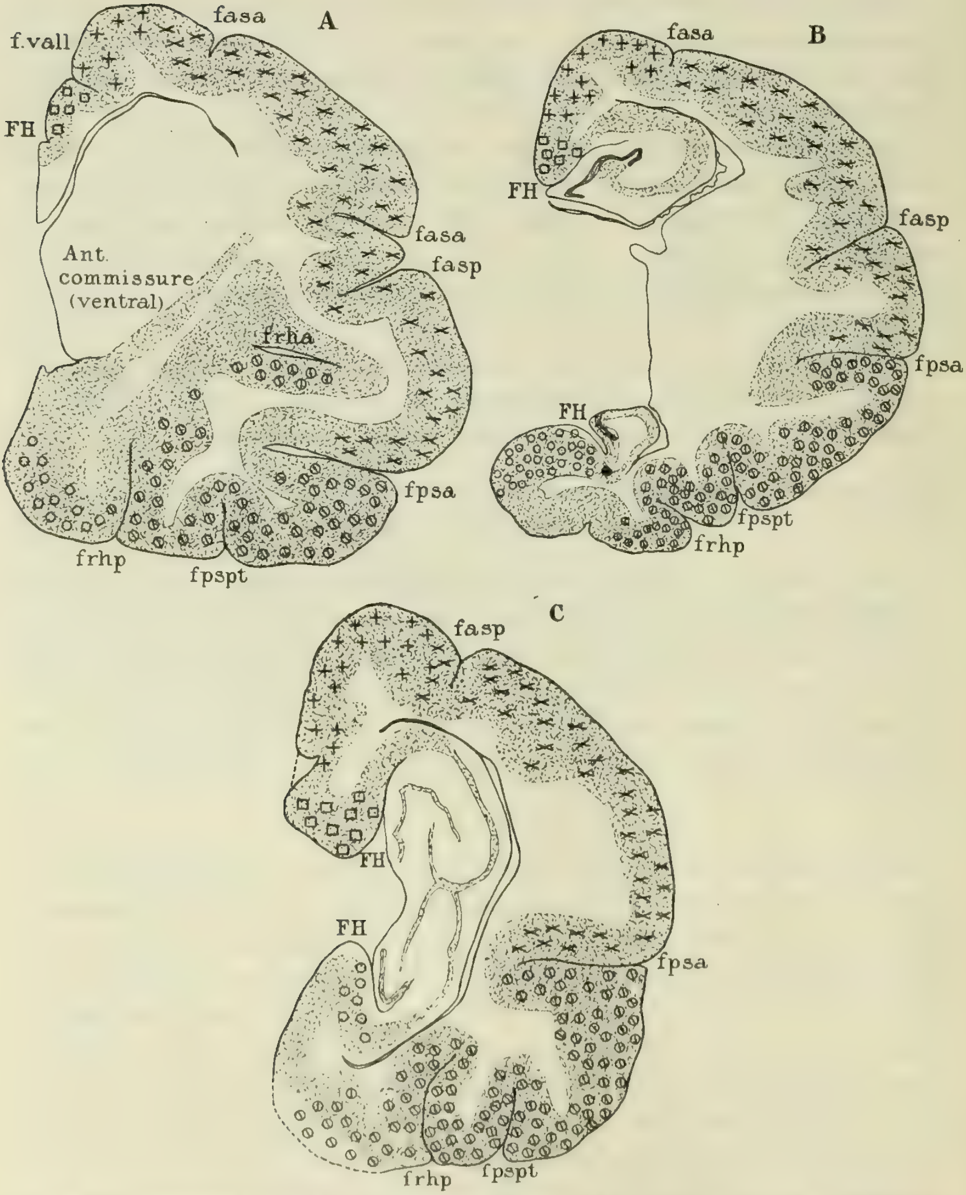
fasa
A
f.vall
FH
fasa
fasp
Ant. commissure (ventral)
frha
fpsa
frhp
fpspt
fasa
B
FH
fasp
fpsa
FH
fpspt
frhp
C
fasp
FH
FH
fpsa
frhp
fpspt

Fig. 2.—Transverse sections of right hemisphere. × 5¼. (For position of sections and lettering *vide* fig. 1.)

TYPE II. + + + (*vide* fig. 3).

Fig. 3 is drawn from the superomesial border. The depth of cortex is here about 1·5 mm. The following layers can be distinguished:—

1. Molecular layer, depth 0·05 mm.

2. A superficial layer of somewhat closely-packed cells of irregular shapes, characterised by being broad relative to their length. The size of these is about 8 to 18 μ in length and 7 to 12 μ in breadth. About 0·1 mm. from the surface the cells begin to get less crowded, and at a depth of 0·2 mm. a layer (3), somewhat poor in cells, succeeds. This is about 0·25 mm. broad. What cells are contained in it seem a shade larger than those of layer 2, and they become larger as one goes deeper. They are somewhat irregular in shape, but many are roughly pyramidal.

4. Layer of large pyramids starts 0·5 mm. from the surface and stretches downwards to a depth of 0·9 mm. The majority of the cells in this layer are elongated and somewhat irregular pyramids measuring up to 30 μ in length and 15 μ in breadth, with an average size of 25 × 10 μ or so. Their nuclei are 7 × 13 μ or somewhat less. Among these large characteristic cells, which are present in considerable numbers, lie many much smaller cells. These granules extend throughout the cortex except in the most superficial portion, but they are most numerous among and immediately below the large pyramids.

5. The rest of the cortex is made up of small polymorphic and spindle-shaped cells measuring about 25 × 8 μ, with roundish nuclei 5·7 μ in diameter.

Distribution.—Type II may be found on the mesial surface of the hemisphere dorsal to the fissura vallaris. Anteriorly it extends at least as far as that fissure, while posteriorly its limit appears to be the radial fissure continuous with fissura postsylvia anterior (*fpsa*). Above it spreads over the superomesial border on to the lateral surface, where it abuts on the area covered by Type III.

TYPE III. × × × (*vide* fig. 4).

Fig. 4 is drawn from a strip on the lateral surface about 5 mm. from the superomesial border, and 3 mm. from the point at which Type II changes into Type III. The depth of the cortex is here about 1·75 mm.

The following layers may be recognised:—

1. *Molecular.*—0·13 mm. in breadth, or more than double the breadth of the molecular layer of Type II.

2. Layer of "small pyramids" somewhat elongated in shape, measuring 12 to 30 μ in length × 5 to 10 μ in breadth.

Although some might correctly be described as pyramids, the majority are not pyramidal, but rather sausage- or spindle-shaped. They often have two apical processes. This layer extends to a depth of about 0·4 mm.

3. A zone about 0·5 mm. in breadth rather poorer in cells than layer 2.

The cells are some of them irregular pyramids slightly larger than in that layer, while others are smaller cells of the granule type. The latter are to be met with also in the deeper layers, but are more numerous here.

4. Layer of large pyramids about 0·4 mm. in breadth.

The cells are much the same as those of the corresponding portion of Type II; they lie 0·4 mm. farther from the surface.

5. Layer of polymorphic cells. This is about three-quarters of the breadth of the corresponding layer in Type II, measuring 0·45 mm. across. The constituent cells of both types appear to be much the same in structure.

Distribution.—This type of cortex covers the greater part of the lateral surface of the hemisphere. Above it changes more or less suddenly into Type II; below and behind the fissura postsylvia anterior (*fpsa*) forms its boundary, while in front it extends almost as far as the fissura rhinica anterior (*frha*). It is separated from the upper lip of that fissure by a narrow strip of cortex of simple structure, rather resembling Type I Type III varies somewhat in its details in different parts of the wide area which it covers. It is narrower on the walls of the fissures, the cells are less numerous and are shorter and broader.

Type IV. ⦶ ⦶ ⦶ ⦶ (*vide* fig. 5).

The depth of cortex is about 1·8 mm. The following layers may be distinguished:—

1. Molecular layer, 0·2 mm. deep.

2. Dense layer of cells of various shapes, measuring 15 to 30 μ in length. There are some pyramidal, giving off one process towards the surface, while others are inverted pyramids giving off two or more processes in that direction; others again are stellate or fusiform. This layer stretches to a depth of 0·5 mm. In its deeper portion, the cells are less crowded, and a number of granules are present.

3. A distinct layer of granules, 0·3 mm. in width, which stretches to a depth of 0·8 mm. The exact shape of the granules was difficult to determine owing to bad preservation. Among them are larger cells of pyramidal or irregular shape.

4. A layer of large pyramids and polymorphic cells, of which many are 30 to 35 μ in length. Among them are smaller cells of the same shapes and scattered granules. This layer has no very definite boundary, but its constituents get smaller at a depth of about 1·1 mm.

Type V. ○○○○ (*vide* fig. 6).

1. The molecular layer is very broad, stretching downwards to depth of 0·4 mm. below the surface.

2. A dense layer of large, darkly staining cells, measuring up to 35 × 20 μ. These cells are characterised by the strong development of their dendrites, two of which are often directed towards the surface.

3. At 0·6 mm. below the surface, or just above, these cells give place to others, distinguished from them by being slightly smaller and irregularly pyramidal or pyriform in shape, with only one apical process. This layer extends downwards to depth of rather less than 0·9 mm.

4. A stratum almost devoid of cells, about 0·1 mm. broad.

5. At a depth of 1 mm. a layer of polymorphic cells may be found, which measure about 20 × 15 μ. Cells of this type occur throughout the rest of the cortex, but at a depth of 1·2 mm. they become less numerous and are partly replaced by numbers of small stellate cells.

6. Thus a sixth layer can be distinguished, characterised by the presence of the latter. It stretches from 1·2 to 1·6 mm. from the surface.

Distribution of Cortex of Type IV and Type V.

Cortex of Type IV may be seen in Section A, lying between the fissura rhinica posterior (*frhp*) and the fissura postsylvia anterior (*fpsa*). In this section the fissura rhinica anterior (*frha*) is cut in a plane more or less parallel to the direction in which it runs but at right angles to its walls. The walls are thus shown in section near to the bottom of the fissure. It will be seen that the cell lamination of the lower wall is of Type IV. In a section taken a little in front of this the whole length of *frha* is cut in this way and the fissure is shown opening on to the lateral surface of the hemisphere, but in such a section the lower wall is made up, at any rate near its outer end, of cortex of Type V. In Section A the latter type of cortex is seen lying on the mesial side of *frhp*. In this region and anterior to it, it is of a slightly different structure to that illustrated in fig. 6, which was taken from Section B. The cells of the outer layer are smaller, not so numerous, nor so rich in dendrites. In Section B it will be seen that Type V has left the region of *frhp* and only covers the part of the rhinencephalon lying next to the hippocampal fissure (*FH*). Meanwhile Type IV has encroached beyond *frhp*, which in Section A formed the boundary between the two types. Types IV and V do not pass directly into one another, but are separated by a broad strip of cortex more or less intermediate in structure. This lacks the most characteristic feature of each of the two types which it separates, namely the layer of granules of the one and the large, darkly staining cells in the outer layer of the other. The latter do not stop suddenly nor die out gradually, but they become first reduced in numbers, then arranged in groups separated by free spaces and then finally disappear.

In Section C the same general arrangement is seen, but the strip of Type V has become narrower and of Type IV broader. In Sections A, B, and C the upper boundary of Type IV is formed by *fpsa*. A short way behind Section C this fissure changes its direction, running more vertically upwards. Cortex of Type IV runs upwards as a narrow tongue behind it.

The Numbers of Nerve Fibres in the Cranial Nerves of Echidna.

It was suggested to me by Dr. Mott that the numbers of fibres in the cranial nerves might offer a useful indication of the relative development of different parts of the cerebrum, and consequently the piece of work described here was undertaken.

The material available was the brain from which the foregoing description of the cerebral cortex was made, and a brain of *Echidna setosa* which had been brought back by Mr. Geoffrey Smith from Tasmania. The latter was not in a good enough state of preservation to be used for any other purpose but the counting of the fibres in the cranial nerves.

Nerves III, IV, VI, VII, IX, X, XI, XII were small enough to be viewed in one field of the microscope under a magnification great enough to see each fibre in the stained transverse section separately, or, when not small enough, they were divided into separate nerve bundles, each of which could be viewed as a whole. The counting of the fibres was in these cases a simple and direct, though somewhat laborious matter. A plan was made of each nerve by means of the "Abbé" drawing apparatus, a dot or circle representing each nerve fibre. The dots or circles were then carefully counted.

Nerves II, V, and VIII were too large in transverse section to be treated in this way, and were not conveniently divided up by connective tissue. Another method had therefore to be adopted. The transverse section of the nerve was placed under a power just low enough for it to be entirely included in the field of the microscope, and its outline was traced by means of the drawing apparatus. Then, without shifting the paper or drawing apparatus, or changing the lenses of the microscope, a Zeiss object netz-micrometer was substituted for the microscope slide bearing the section, and the small squares ruled on this apparatus were traced on the top of the drawing. Each of these squares has sides 1/20 mm. in length, and therefore an area of 1/400 sq. mm. By counting the number of squares included in the outline of the nerve, a fairly accurate estimate of the area of the transverse section can be obtained, which is not in any way affected by the distortion produced by the camera lucida.

A sufficiently high power was then put on the microscope and the outline of a convenient number of squares traced on a fresh piece of paper; the section was then substituted for the micrometer and all the fibres lying within the outline of the squares were marked. Thus the number of fibres in a definite area of the section was ascertained. This was done two or three times in different parts of the nerve. By dividing the total area of the nerve by the area for which the number of nerve fibres is known and multiplying by the latter number, an estimate of the total number of fibres in the nerve may be obtained, the accuracy of which will vary with the ratio of the known to the unknown, and with the degree of uniformity with which the fibres are scattered throughout the nerve.

The following is a concrete example, namely the optic nerve in Series A :—

Under the low power (16 mm. appochr. obj., 8 comp. occ.), its area was found to be 0·295 sq. mm. The number of nerve fibres for 0·01 sq. mm. was found by the high power (2 mm. appochr. imm. obj., 4 comp. occ.) to be in three different places 974, 942, 992, respectively, which have an average 969.

$$969 \times 0{\cdot}295 \div 0{\cdot}01 = 28{,}585.$$

The results obtained by these two methods are summarised in the table.

Table showing Number of Nerve Fibres in the Cranial Nerves of two Specimens of Echidna.

Nerve.	Brain A.	Brain B.
II	28,585*	46,566*
III	1,978	1,849
IV	404	223
V	—†	43,410*
VI	671	474
VII	4,281	3,535
VIII	24,565*	23,523*
IX	2,740	2,170
X	3,617	3,042
XI	3,227	2,180
XII	5,214	2,865

A, Echidna from Zoological Gardens. B, *Echidna setosa* from Tasmania.

* Number estimated by the method described in text.

† Owing to there having been only a very short piece of the Vth nerve in Specimen A available for sectionising, which was about as long as it was broad, it was accidentally cut longitudinally and not transversely, and was thus useless for the purpose of counting the fibres.

Cortical Lamination and Localisation in the Brain of the Marmoset.

By F. W. Mott, M.D., F.R.S.; E. Schuster, D.Sc.; and W. D. Halliburton, M.D., F.R.S.

(Received October 20,—Read December 9, 1909.)

[Plates 6 and 7.]

The following research is one which has been carried out on lines similar to that previously published by two of us* in relation to the brain of the Lemur. A series of sections of the cerebral cortex has been examined in order to map out the extent and boundaries of the types of cell-lamination observed. It is now well known that these differences are correlated with differences in function, and this method of histological localisation of function (as it may be termed) has been controlled by the physiological method of stimulation. The histological portion of the work has been carried out by two of us (M. and S.), and the physiological experiments were performed at King's College, London, by the third (H.).

Brief Introductory Remarks upon Correlation of Structure and Function.—The smooth brain of the Marmoset stands in structural development in some respects midway between the smooth brain of the Lemur and the convoluted brain of platyrrhine Apes higher in the zoological scale, *e.g.* Macacus. There are certain facts in the morphology, mode of life, and habits of these three types of animals which may be correlated with the differences in structure of their brains. The Lemur is an arboreal animal, and being nocturnal and insectivorous, it depends largely upon smell as a guiding sense. It is not surprising, therefore, to find large olfactory nerves and a relatively large area of archi-cortex. In the Lemur (half ape) the neo-cortex has not developed sufficiently to push the rhinal fissure downwards to the under surface of the temporal lobe as is found to be the case in the Ape.

The eyes in the Marmoset are set so that the visual axes are parallel. The optic nerves are well developed, and, according to Lindsay Johnston, there is a macula; it may, therefore, be assumed that this animal possesses binocular vision, which enables it to employ this sense for obtaining food by seizing with its mouth the insects and fruits upon which it lives.

In contrast with the Lemur, the sense of smell plays a subordinate part in the preservation of the animal, consequently the olfactory nerves and the archicortex are relatively less developed, but the occipital cortex is more developed, and forms a definite occipital lobe covering the cerebellum. The relative extent of the motor area and tactile motor or kinæsthetic area of the neo-cortex of the Marmoset, compared respectively with that of the Lemur and with that of the *Macacus rhesus,* shows that it corresponds more with the former than with the latter. This fact may be correlated with the following statement by Bates concerning the habits of the Marmoset: "they are arboreal animals, but their manner of climbing resembles that of squirrels rather than monkeys; this is due to the fact that they have no means of gripping a bough since the pollex is not opposable, neither is the tail prehensile. They therefore confine themselves to the larger branches, where their long claws are of assistance in enabling them to cling securely to the bark." The Marmosets, owing to their claws and non-opposable pollex, are unable to use their limbs for prehension with anything like the delicacy and refinement of the higher Apes. Although the Marmoset possesses stereoscopic vision, it is unable to translate all visual into tactile-motor impressions to the same degree of perfection as the Macacus, consequently the stereognostic sense must be comparatively rudimentary. This fact may account for the existence of a convoluted parietal lobe which indicates a considerable extension of surface lying between the occipital lobe and the post-central convolutions in the Macacus, which is not found either in the Lemur or in the Marmoset.

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* Mott and Halliburton, 'Roy. Soc. Proc.,' B, vol. 80, p. 136. See also Mott and Kelley, *ibid.*, p. 488, 1907.

GENERAL DESCRIPTION OF THE BRAIN.

The Marmoset has a small brain, and the surface is broken by unusually few fissures. On the external surface the Sylvian fissure (F. S., fig. 2) is the most conspicuous, and there is also an indication of a lateral sulcus (f. pa.) on the temporal lobe. On the under side of the temporal lobe the rhinal fissure (f. rh.) is found, and it may be noticed that it does not come so far around as in the Lemur, where it is seen on the external surface. Thus in the Lemur there is a larger space between the hippocampal and the rhinal fissures, and, therefore, a more extensive olfactory area than in this animal. On the orbital surface a small orbital sulcus appears. An unbranched calcarine fissure (f. cal.) is seen on the mesial surface extending from the hippocampal nearly to the extremity of the pole. A small hippocampal tubercle lies in front of this hippocampal fissure, and a short intercalary sulcus appears above the corpus callosum, while in the frontal region there is an indication of a small fissure. These sulci, however, are only faintly indicated on the surface of the brain, and only the Sylvian, the hippocampal, and the calcarine fissures show any considerable depth in section. It will also be observed that there is a definite occipital lobe which covers the cerebellum.

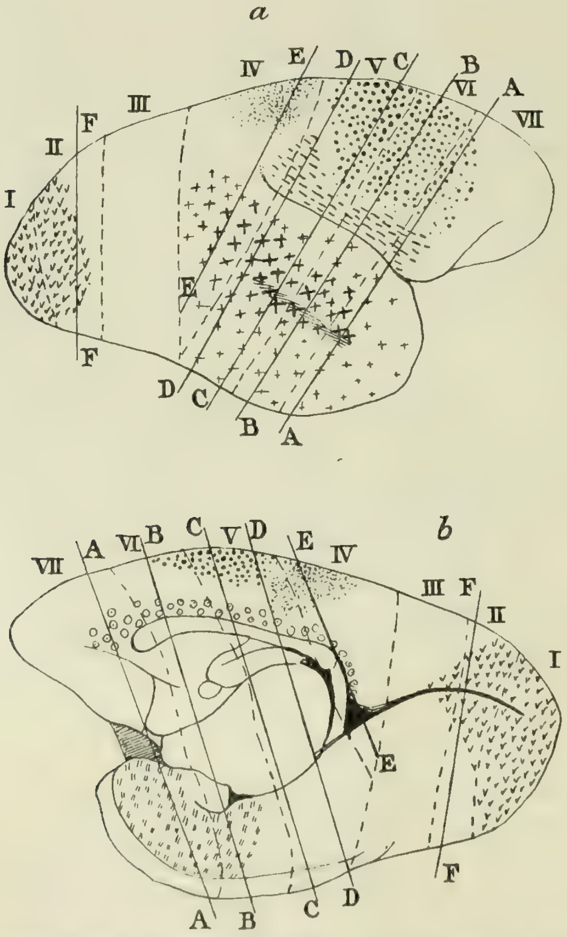

Fig. 1.—Right hemisphere of Marmoset's brain, seen (*a*) from the outer and (*b*) from the mesial surface, × 2. The extent of the different types of cortex described is shown diagrammatically (see key). The lines AA, BB, etc., show the planes of the sections represented in fig. 2. The Roman figures refer to the paraffin blocks made, and have no significance except to the authors.

Neopallium. Motor Area A (*fig.* 3).* Extent and Boundaries.—The motor type of cortex is found, as might be expected, on the front half of the brain, and above the Sylvian fissure (see fig. 1). The greater part lies on the external surface, but it also spreads over on to the mesial. On the mesial surface, where the post central and limbic types adjoin it, it is not difficult to determine the border line, but anteriorly and posteriorly, and inferiorly on the dorsal surface, the type becomes less characteristic, and gradually its distinctive character is lost as it merges into the intermediate indefinite areas which surround it. The lamination is seen in its most typical form in the posterior part of the area, near the postero-mesial border.

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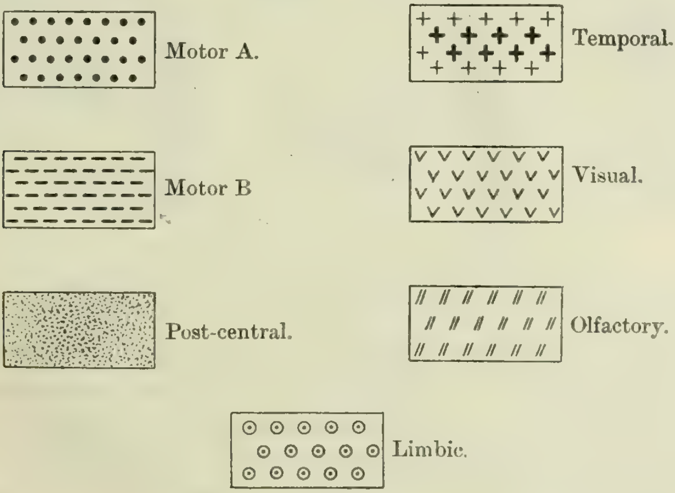
* For figs. 3 to 6 see accompanying plates (Plates 6 and 7).

Characteristics.—The cortex measures about 1·5 mm. in depth, the molecular layer 0·1 mm., the pyramidal layer about 0·75 mm., the zone in which the Betz cells lie about 0·2 mm., and the polymorph layer 0·3 mm. to 0·4 mm. The pyramidal cells are rather larger and less regularly and closely arranged than in most other parts of the neopallium. Scattered granule cells are found among them, but they do not form a separate layer. Below the pyramids, lying in a pallid zone, there is a well-marked line of Betz cells, the largest of which measure from 50 μ to 60 μ. These Betz cells, and the absence of a granule layer, are the most marked characteristics of this area.

Motor Area B (*fig.* 4) lies below the motor area A between it and the Sylvian fissure. On stimulation, movements of the jaw and mouth occur as described in the account of the stimulation experiments, but it shows a marked difference to the type just described in its histological details. The molecular layer measures 0·1 to 0·15 mm., the pyramidal layer about 0·75 mm., the pallid zone about 0·1 mm., the granule layer 0·3 to 0·4 mm., and the polymorph layer about 0·3 mm. The presence of a distinct band of granules specially distinguishes it from the cortex above. There is also



Key to diagrammatic representation of different types of cortex in figs. 1 and 2.

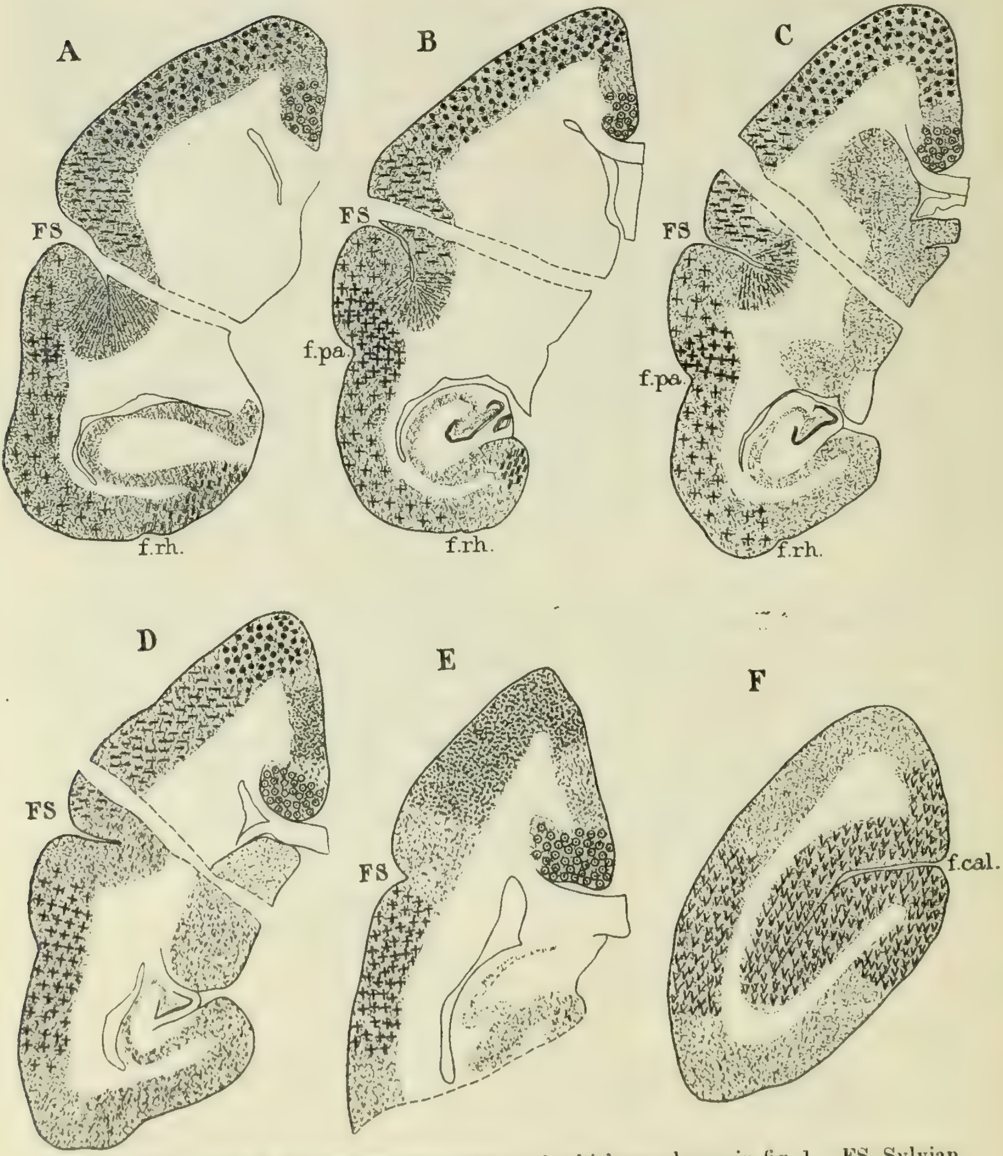


Fig. 2.—Diagrams of six sections, the position of which are shown in fig. 1. FS, Sylvian fissure ; f.pa., lateral fissure ; f.rh., rhinal fissure ; f.cal., calcarine fissure.

a disappearance of the large Betz cells, the largest cells in this area measuring from 25 to 30 μ. It has indeed the appearance of a sensory area, and it is possible that it is a continuation of the post central area. The fact, however, that it was from this part of the hemisphere that movements of the face, jaw, mouth, and tongue were obtained, renders it probable that this is a sensori-motor area. Experiments on the Lemur also showed that movements of the head and eyes, mouth, side of face and tongue and pricking of ears were obtained by stimulating a cortex similar in position and structure; though in the case of the Lemur the essentially sensory type of the cortex, though recognisable, was not so pronounced.

Post Central Area (*fig.* 5). Extent and Boundaries.—The post central type lies behind the motor, covering only a relatively small external area; but on the mesial surface it spreads out and extends farther down than the motor type, and is found wedged in between the posterior part of the motor and limbic areas. These boundaries are only approximate, as it is extremely difficult to distinguish the peri-focal area of this type of cortex from the surrounding intermediate types.

Characteristics.—The depth of the cortex is about 1·4 mm.; the molecular layer measuring about 0·12 mm., the pyramidal layer about 0·6 mm., the granules 0·15 mm., and the polymorph layer about 0·4 mm. The molecular layer is slightly deeper than in the motor area, and the pyramids as a whole are rather smaller, more regular, and more numerous. There is a distinct band of granules which distinguishes this area from type motor A, and it may be noticed that there is no distinct pallid zone, such as that in which the Betz cells lie. The pyramidal cells above the granules are not of remarkable size, but a characteristic line of large plump infra-pyramidal cells is conspicuous, the largest measuring about $35\,\mu$ to $20\,\mu$.

Temporal Area (*fig.* 6). Extent and Boundaries.—A temporal type of cortex probably covers the temporal lobe from the Sylvian fissure on the external surface to the rhinal fissure on the underside. It is bounded superiorly by the Sylvian fissure, anteriorly by the rhinal fissure. On the under surface and posteriorly it merges gradually into the intermediate areas lying between the temporal and visual types. But the cortex is only seen in its most typical form on the external surface below the Sylvian fissure, as marked on the diagram by the blacker crosses (fig. 1).

Characteristics.—The depth of the cortex is about 1·5 mm., the molecular layer measuring 0·12 mm., the pyramidal 0·6 to 0·7 mm., the granules about 0·25 mm., and the polymorph layer about 0·5 mm.

In the most typical region the cells of the pyramidal layer, like those in the motor area, are of relatively good size, and are neither very numerous nor regular in arrangement as compared with other regions. The most conspicuous feature of this area is the presence of large, attenuated, deeply staining pyramidal cells, the biggest measuring about $40\,\mu$ by $10\,\mu$. These are found both above and below the granules, but are more numerous above. The granule layer is deep and very rich in cells. In its peri-focal area the cortex becomes less typical. As it approaches the anterior extremity of the temporal lobe a comparative poverty of cells is noticeable, both in the pyramidal and granule layers. Posteriorly the pyramids are smaller and more crowded, and in both regions the large pyramids are greatly diminished in size.

Visual Area. Extent and Boundaries.—The visual area covers the posterior pole of the hemisphere. It extends forward on the external surface for about 3 or 4 mm., and on the mesial surface to about halfway between the pole and the corpus callosum, as shown in fig. 1.

Characteristics.—The depth of the cortex is about 1·5 mm., the molecular layer measuring about 0·1 mm., the pyramidal layer about 0·6 mm., the granule layer 0·3 mm., the line of Gennari 0·1 mm., and the polymorph layer 0·3 to 0·4 mm. The pyramidal cells are rather smaller than those of the motor and temporal types, and more numerous, and the granules also are smaller and more crowded. Large solitary cells of Meynert, measuring about 20 μ by 15 μ, are scattered above and below the granules.

In the Lemur the visual cortex is more shallow, and the individual cells are smaller and more closely crowded together.

Archipallium. Olfactory Area. Extent and Boundaries.—The olfactory area lies on the under side of the anterior portion of the temporal lobe, the rhinal fissure forming its inferior and anterior, and the hippocampal fissure its superior boundary. Posteriorly it extends to about the level of the extremity of the rhinal fissure.

Characteristics.—This cortex measures about 1 mm. in depth, the molecular layer measuring about 0·2 mm., the pyramidal layer about 0·4 mm., the pallid zone about 0·1 mm., and the polymorph layer 0·3 to 0·4 mm. Thus, though the cortex as a whole, and the pyramidal layer particularly, are more shallow than in the neopallium, the molecular layer is deeper. A line of characteristic cells lies at the top of the pyramidal layer, larger than the adjacent pyramids, they are more closely crowded together, and form a characteristic darkly staining line under a low power of the microscope. They are angular, and generally quadrilateral in shape, and have several clearly staining branched processes, of which two generally pass upwards into the molecular layer. The pyramidal cells as a whole are comparatively large, though they are not numerous; but there is no line of specially large supra- or infra-granular pyramids. Scattered granules are seen among the pyramids, but do not form a distinct granule layer. A conspicuous pallid zone lies below the pyramids. The polymorph layer is composed of blunt roundish cells, similar to those found in the limbic area.

Limbic Area. Extent and Boundaries.—The limbic area covers the portion of the cortex lying immediately round the corpus callosum, from its anterior to its posterior genu. Sections cut through the hippocampal fissure show a narrow strip of cortex having the same structure bordering the olfactory type, lying between it and the distinctive structures of the hippocampal fissure. It is probable that, as in the Lemur, a narrow strip of limbic cortex is to be found curving around the posterior genu of the corpus callosum, and folding in to the hippocampal fissure, thus forming a link between the upper and lower portions of the limbic lobe.

Characteristics.—The cortex measures 0·8 mm. to 1·0 mm., the depth of the molecular layer being about 0·2 mm. The remainder of the cortex cannot be separated into layers. The cells immediately beneath the molecular layer have a tendency to be pyramidal in form, but below these the mass of them are blunt, shapeless, and faintly stained, only becoming more angular at the bottom of the cortex, in the position of the polymorph layer.

Localisation of the Motor Area by Stimulation.

The animals were anæsthetised either by ether or A.C.E. mixture, and anæsthesia was maintained throughout the whole of the operation until the animals were killed at the conclusion of the experiment.

In dealing with so small a brain, the parts are so delicate that the greatest possible care has to be exercised throughout. In one experiment the animal died from hæmorrhage owing to the injury of a vessel entering the longitudinal sinus, in an attempt to separate one hemisphere from its fellow in order to stimulate the mesial surface, and further attempts to explore this portion of the cerebrum were therefore abandoned.

In the remaining two experiments, in which the greater part of the external surface of the hemisphere was exposed, we obtained concordant results by the method of stimulation. The stimulating electrodes were finely pointed and very close together; they were made of platinum and were connected to the secondary coil of a Du Bois Reymond inductorium arranged for faradisation. The strength of current employed was that which could just be felt as a faint tingling on the tongue. The accompanying illustration (fig. 7) gives the results of these experiments. It represents the left hemisphere seen partly from the side and partly from above, to show the excitable area. This will be seen to be situated wholly above and in front of the Sylvian fissure. If the various figures placed on the diagram are compared with the description beneath it, it will be seen that the head area is a large one, and above this are situated the centres for the upper limb and then those for the lower limb, which is the usual arrangement.

The larger cortical representation of the head and face region enabled one to differentiate its subdivisions much more thoroughly than was possible in the case of the limb movements. Since in catching insects a quick play of mouth and tongue must be required, this may be an explanation of the large cortical representation of the tongue, mouth, and face. Stimulation of the occipital region gave wholly negative results, although this portion of the brain was well exposed and explored as thoroughly as possible with currents of the usual strength, as well as with currents which were somewhat stronger. There is no doubt from histological examination that the visual area is situated at the occipital pole as in other animals. A similar negative result was obtained on stimulation of the corresponding region of the brain of the Lemur,* and in both cases this is probably due to the distance separating the solitary cells of Meynert in the parts stimulated.

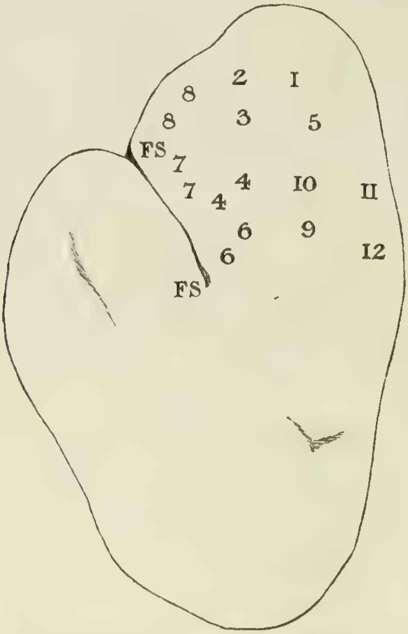


Fig. 7.—Left Hemisphere of Marmoset. Mag. 3·5.

F.S. Fissure of Sylvius. 1. Deviation of head and eyes to opposite side. 2. Opening of eyes. 3. Eye movements. 4, 4. Eye and face movements. 5. Deviation of head. 6, 6. Face movements. 7, 7. Jaw and face movements, including right-sided deviation of mouth. 8, 8. Tongue movements. 9. Extension of wrist to opposite side. 10. Extension of wrist and flexion of arm. 11. Movements of opposite leg. 12. Flexion of opposite hip and knee.

No portion of the cerebral cortex when stimulated give any movements of the external ear; this is in striking contrast to what was found in the Lemur, where the ear area is a large and important one. No experiments of the nature of extirpation were carried out.

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* Mott and Halliburton, *loc. cit.*, p. 140.

Summary.

1. The brain of the Marmoset is small and broken by comparatively few fissures, and of these only the Sylvian, hippocampal, and calcarine fissures show any considerable depth.

2. A series of sections of the cerebral cortex were made to map out the boundaries and characters of the types of cell-lamination which occur. The result of this work is given in the accompanying diagrams, and some of the principal types are illustrated in the plates.

3. As was found in the Lemur, the motor area consists of two types; in one of these (motor area A) the Betz cells are large and conspicuous; in the other (motor area B), which corresponds to face and head movements, not only are the motor cells smaller, but a layer of granules indicates that this part of the brain is sensori-motor in function.

4. By stimulation, the boundaries of the excitable cortex were found to coincide with those mapped out by histological examination. The sequence from below upwards of head, upper limb, and lower limb is that which usually prevails in mammals.

5. Although no movements of the eye were obtainable by stimulating the occipital pole, this is to be attributed to the difficulty of the experiment. Histological examination of this region shows it to possess the structural characters of the visual cortex in other animals.

6. The other portions of the cortex, which are described in full in the text, call for no special comment. They do not differ in any marked character from those previously described in corresponding regions in related animals.

[*Additional Note.*—The cortex of the Marmoset (Hapale) has been mapped out in great detail by K. Brodmann* in a work which has come to our notice only since the completion of our paper.

As this work is rather in the nature of a general summary of the subject of cortical lamination, no special descriptions or figures are given of the numerous types of cell lamination to which he refers. To what extent his results correspond with our own may be seen by a comparison of our fig. 1 with his figs. 96 and 97 (p. 161). It will be noticed that there is a very close agreement with regard to the extent of the visual area. While our "Motor A" corresponds in position fairly closely with his Feldern 4 and 6 (area gigantopyramidalis and area frontalis agranularis), the larger dots which in our figures represent the region in which the largest pyramids may be found are spread over practically the same as his Feldern 4.

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* Dr. K. Brodmann, 'Vergleichende Lokalisationslehre der Grosshirnrinde,' Leipzig, 1909.

None of his types appears to correspond with our "Motor B." But its position at its posterior end is occupied in his figure with the lower end of his area post centralis (Feldern 1 to 3). Our post-central cortex occupies much the same position as his area præparietalis (Feldern 5), except that it does not extend so far downwards on the lateral surface. With regard to the temporal and olfactory regions we are in fair agreement.]

The Caudal Fin of Fishes (*Preliminary Paper*).

By Richard H. Whitehouse, M.Sc. Birm.

(Communicated by Prof. F. W. Gamble, D.Sc., F.R.S. Received October 30,—Read December 9, 1909.)

CONTENTS.

PAGE

I.—Introduction 134

II.—Types of Caudal Fin—

- 1. Protocercy 135
- 2. Heterocercy 136
- 3. Homocercy 138
- 4. Gephyrocercy 142

III.—Morphology of Parts 142

I.—*Introduction.*

The aim of the present paper is to give, as concisely as possible, the summary of two years' work on the caudal fins of fishes and also to revise the definitions relating to the different types of caudal fin. The revision of the definitions of old established names such as protocercy, heterocercy, etc., has proved to be quite essential owing to the vagueness and even errors connected with these terms so much in evidence in text-books of zoology; this vagueness undoubtedly exists, or otherwise such mistakes as attributing diphycercy to such types as the *Gadidæ* and the eels would not occur as they do. I have also been assured by several eminent teachers of zoology that the subject needs clearing up.

The whole of my work has included the examination of well over a hundred different species of fishes representative of nearly all the sub-groups of Teleosts, several Ganoids, and a few Elasmobranchs. Probably the most interesting results have been obtained from the *Gadidæ* and *Apodes* and the frequent misinterpretations to be read on the caudal fin of these groups has convinced me that caudal fins have not been subjected to a sufficiently close structural examination; for this reason I have made a point of examining microscopically every tail fin, and have invariably subjected each one to clearing agents, a process which has revealed many features it is impossible to recognise by mere dissection.

Since this must be considered only a preliminary communication, it should be remarked that the statements here made will be discussed in detail and substantiated by examples in the near future. The work was suggested by, and carried out under the supervision of, the late Professor Bridge, F.R.S.; I would like to take this opportunity of expressing my deepest thanks to many friends who have supplied me with material and given valuable criticisms.

II.—*Types of Caudal Fin.*

It is now generally admitted that the specialised caudal fin of fishes has been evolved from a common type called the protocercal, and that this primitive type was originally part of a continuous median fin. By the upturning of the end of the axis, heterocercy was established, the form exemplified by modern Elasmobranchs and Ganoids. Specialisation of the heterocercal form produced the homocercal, as seen in the adult Teleost.

1. *Protocercy.*—A protocercal fin may be defined as one which is primitively symmetrical externally and internally. This implies that the tail has not undergone any reduction or modification of the original form.

It is probably correct to say that no true fishes possess a protocercal tail in the adult condition; the Cyclostomes are generally credited with having protocercal tails, but it is likely that even here a degenerate condition is present. It will be well to state clearly what is meant by an internally and externally symmetrical caudal fin. Internal symmetry depends on the endoskeleton; the chorda must end in a perfectly straight line, and the supports of the fin-rays must be equal in number and kind dorsally and ventrally; thus the dorsal and ventral fin-rays contributing to the caudal fin must be equal in number and similarly supported. External symmetry obviously depends on the equal size of the dorsal and ventral lobes of the fin.

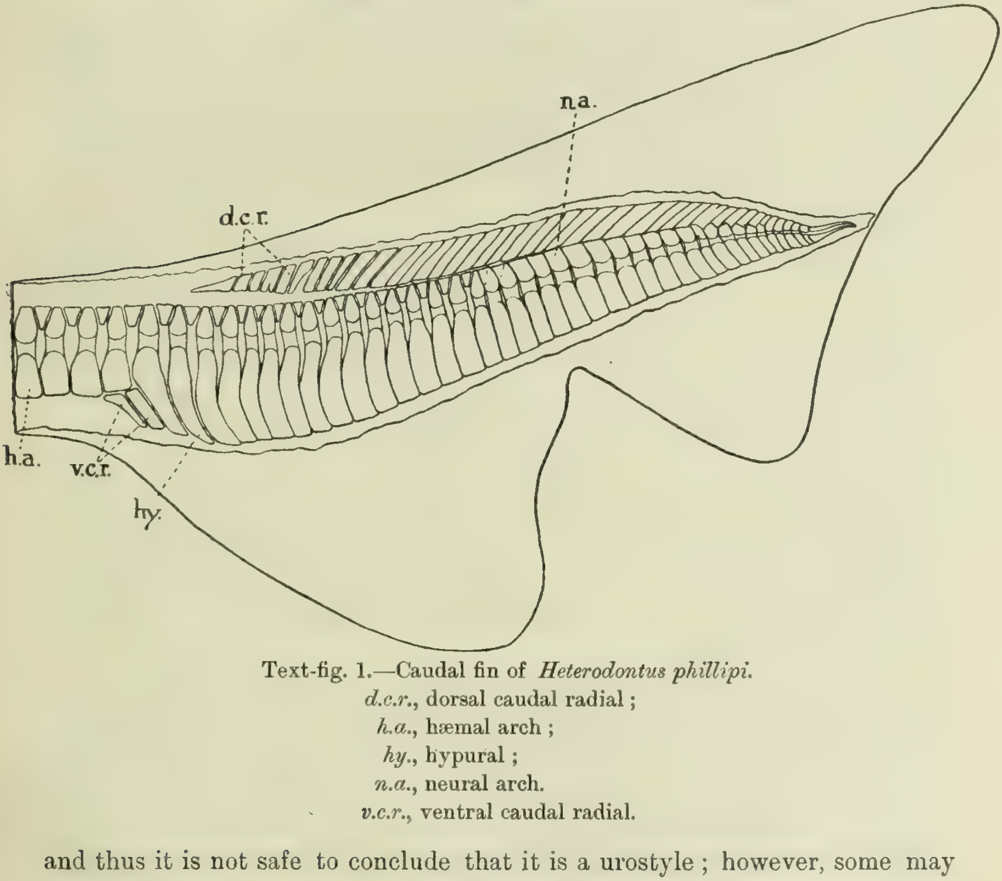
Unfortunately, the terms protocercal and diphycercal have come to be regarded by authors as synonymous; strictly speaking, the term diphycercal applies to a caudal fin which is quite symmetrical externally and internally, with no qualification regarding its primitive or secondary symmetry; thus it is a little vague. Primitive diphycercy is synonymous with protocercy, and secondary diphycercy with gephyrocercy, which will be dealt with later. If this distinction were always kept in mind when speaking of caudal fins, much inconvenience would be avoided. For all practical purposes, the term diphycercy may be dismissed; it may, however, be retained as applying to a caudal fin which it may not be wise or possible to refer to as protocercal or gephyrocercal, owing to lack of definite knowledge regarding its primitive or secondary nature.

Since all fishes first possess a protocercal tail in the embryonic condition, it seems quite safe to infer that it is the true primitive form. As all the complications of the specialised caudal fin of Teleosts have to be evolved from this primitive type, one of the first questions which arises is,—How were the fin-rays of the protocercal tail supported? It is probably impossible to say; but since the caudal fin formed only a part of an undifferentiated median fin system, it is not unreasonable to conclude that they were supported in a manner exactly similar to that by which the fin-rays of the rest of the median fin were supported; now all the rays of the median fins, with the exception of the caudal, in modern fishes, are invariably supported by radials or somactids, hence it may be that radials were once the supporting elements in the caudal. Embryology lends little aid in substantiating this, because before development has proceeded far, the shape of the caudal becomes modified into the heterocercal form.

As a final word regarding protocercy, it must not be supposed that the term is always associated with a continuous median fin; during development, in Teleosts which have differentiated median fins, the elements of the permanent differentiated fins are laid down before heterocercy is established, so that there is evidence for supposing that differentiation occurred before heterocercy was adopted.

2. *Heterocercy.*—A heterocercal caudal fin may be defined as one in which the extremity of the chorda is directed upwards, and as a consequence is unsymmetrical externally and internally, the ventral lobe being greater than the dorsal; and, moreover—and this is a most important feature, to which I have not seen attention drawn—when centra are formed, these elements remain distinct to the end of the axis.

To each of these three features exceptions will be found; for example, many heterocercal tails are of slender build and somewhat lash-like, and consequently there is frequently no appreciable upturning of the axis; *Chlamydoselachus* is a case in point, and often the common dog-fish illustrates the same thing. Regarding the externally unsymmetrical shape, exceptions are much rarer, but *Amia* may be considered to have an externally symmetrical tail-fin; the external form of *Polyodon*, too, is a very close approximation to symmetry; however, internal asymmetry seems to be universal in these cases. The condition concerning the presence of distinct centra to the end of the axis seems to have several exceptions, *e.g.*, *Heterodontus*; a close examination of the extremity of the axis will show a comparatively elongated cone equal in length to several of the preceding vertebræ; this shows no sign of having been separate vertebræ previously, and thus it is not safe to conclude that it is a urostyle; however, some may prefer to regard it as an incipient urostyle, but it never occupies anything but a very insignificant part of the upturned axis.



Text-fig. 1.—Caudal fin of *Heterodontus phillipi.*
d.c.r., dorsal caudal radial;
h.a., hæmal arch;
hy., hypural;
n.a., neural arch.
v.c.r., ventral caudal radial.

Concerning the endoskeletal supports of the heterocercal caudal fin, on the dorsal side, immediately above a series of neural arches, is a series of radials (text-fig. 1, *d.c.r.*) supporting the fin-rays; for the greater part of the caudal fin, these radials remain distinct from the arches, as do those of the dorsal fin in front, though to a greater extent, but towards the extremity they fuse with them to form a single cartilage. On the ventral side the fin-rays are supported for nearly the whole length of the fin by cartilages directly connected with the vertebral axis; but at the anterior end of the fin there are frequently a few radials quite distinct from the hæmal arches, and supporting fin-rays; immediately following, the radials are seen to have fused with the arches, and the line of fusion is often retained. The caudal fins of *Galeus* and *Acanthias* are good examples. Since I believe the majority of the ventral fin-ray supports to be double structures, formed by the fusion of radials with hæmal arches, I have called them hypurals.

The caudal fin of *Amia* has been called hemi-heterocercal; it is perhaps the most highly specialised of heterocercal tails and closely approaches the homocercal of the Teleosts. For this reason the term hemi-homocercal seems to me a more suitable term. But it has an essential feature of heterocercy, namely, the retention of individual vertebræ to the end of the vertebral axis.

3. *Homocercy.*—Before entering on this type of caudal fin it will be necessary to call attention to a proposed modification of nomenclature with respect to three parts of the endoskeleton. Full reasons will be given later, and I will here merely define the terms as understood in the present paper.

Any epaxial element, which is directly connected with the vertebral axis, and which supports one or more fin-rays distally, will be called an epural or epural bone. In the second place the structures usually spoken of by previous authors as epurals will here be called dorsal caudal radials; they consist of free slender bones situated epaxially, supporting fin-rays at their distal ends. The third term to draw attention to is "last vertebral segment," which will be used to imply the last vertebra together with the urostyle if such is present.

The homocercal caudal fin, which is characteristic of the Teleostei, is to be considered a specialised form of the heterocercal type. The term homocercal is very broad in application; however, a caudal fin which is externally symmetrical, but in which the majority of the fin-rays are supported internally by hypaxial elements, and in which a urostyle is present either in the adult or at some time during larval history, may safely be referred to as a homocercal caudal fin.

Except for two genera, *Fierasfer* and *Orthagoriscus*, mentioned by Ryder* as gephyrocercal, it is probable that there is no case among the Teleostei, where a caudal fin is present, in which the tail is not homocercal.

The aim of the present remarks is merely to present to the reader three forms of the homocercal fin, in order to illustrate the process of specialisation in this type, and to state very briefly conclusions as to the morphological value of the endoskeletal supports of the fin-rays.

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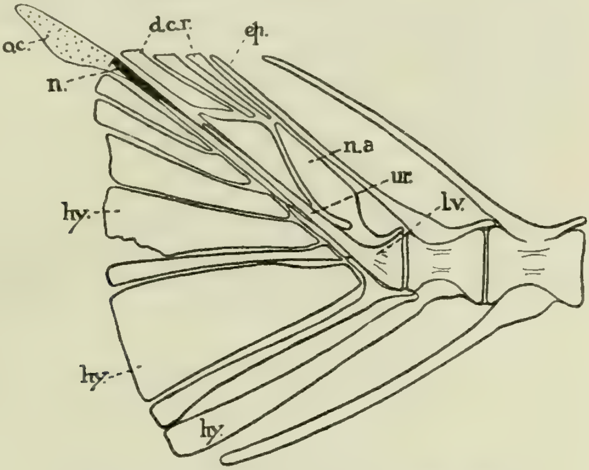
* J. A. Ryder, "Evolution of the Fins of Fishes," 'Rep. U.S. Comm. of Fish and Fisheries,' 1884, pub. 1886.

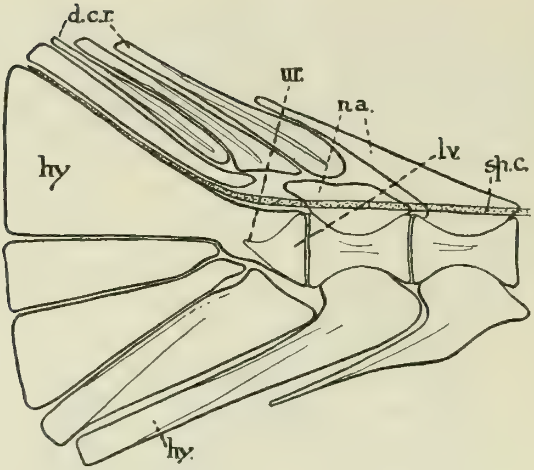
(a) *Clupea pilchardus* (*Malacopterygii*).—The caudal fin of this fish is taken as illustrating one of the least specialised of homocercal forms.



Text-fig. 2.—Caudal fin of *Clupea pilchardus.*
d.c.r., dorsal caudal radial ;
ep., epural ;
hy., hypural ;
l.v., last vertebra ;
n., notochord ;
n.a., neural arch ;
o.c., opisthural cartilage ;
ur., urostyle.

A careful reference to the above figure will render a full description unnecessary ; the points to which special attention is drawn are (1) the presence of a pronounced urostyle ; (2) the presence of the notochord beyond the end of the actual vertebral column ; (3) the large number of hypural bones, viz., seven, associated with the last vertebral segment ; and (4) the presence of an epural bone to the penultimate vertebra. A careful comparison of a large number of Teleostean caudal fins has led me to regard these as the features which characterise a lowly specialised homocercal caudal fin.

(b) *Trigla lineata* (*Acanthopterygii*).—The caudal fin of *Trigla lineata* illustrates a fairly intermediate type between the lowly and highly specialised homocercal form.



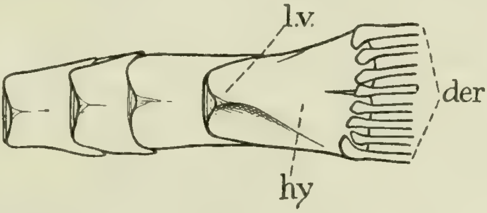
Text-fig. 3.—Caudal fin of *Trigla lineata* as seen after clearing.
d.c.r., dorsal caudal radial ;
hy., hypural ;
l.v., last vertebra ;
n.a., neural arch ;
sp.c., spinal cord ;
ur., urostyle.

The above figure shows the structure of the endoskeleton as it appears after the bones have been cleared in xylol or turpentine, for, only by so doing can the exact structure be followed.

Now by comparing the features here figured with those of *Clupea*, it will be seen in what direction specialisation has been at work. (1) The whole of the exserted notochord has been aborted; this implies considerable abbreviation. (2) The urostyle is no longer a well-marked independent structure, but is reduced almost to extinction, and has become incorporated with a hypural bone ; this is one of the most important effects of specialisation. (3) Another point of difference from the preceding type is the presence of four hypurals, in the place of seven, to the last vertebral segment ; concentration has occurred, the smaller number being most probably due to the lateral fusion of several ; this reduction in the number of hypurals to the last vertebral segment has the effect of producing much greater rigidity and hence usefulness. (4) Although not an invariable rule, a reduced neural arch to the penultimate vertebra, as exhibited in *Trigla*, is usually associated with a specialised homocercal caudal fin ; no satisfactory explanation of this can as yet be given.

(c) *Lophius piscatorius* (*Pediculati*).—An extreme type of specialised homocercy is illustrated by the caudal fin of *Lophius*.

All the dermotrichia are hypaxial, to the total exclusion of the dorsal contribution to the fin. There is practically only one hypural, although this is cleft distally, showing it to be composed of at least two; the last vertebral segment and the hypural become one bone, and indistinguishably fused together. The spinal cord can be traced to the end of the hypural bone. A more specialised type of homocercy can scarcely be expected.



Text-fig. 4.—Caudal fin of *Lophius piscatorius*.
der., dermotrichia cut short;
hy., hypural;
l.v., last vertebra.

In the three preceding types, a broad outline of the effects of specialisation in homocercal tails is shown, and it will be seen that great abbreviation of the axis and restriction of the caudal fin to hypaxial rays are among the chief. Abbreviation is demonstrated by the excessive reduction of the urostyle and the consequent concentration of the supporting elements of the rays; but also—and this is a point which does not seem to have been noticed before—by the presence of the spinal cord beyond the actual termination of the vertebral axis; this last feature seems to be universal in instances of more advanced homocercy.

Practically all the gradations between the three forms of homocercy described are to be found among other Teleosts. Special attention has been directed to ascertain whether the various grades in specialisation corresponded with the general classification which is based on specialised features in general, with a view to discovering whether the structure of the caudal fin could be used as a factor in classifying fishes. It has been found, however, that the successive grades of specialisation in the caudal fin do not correspond with the order of classification given by Boulenger in the Cambridge Natural History; and hence it may be said that, in general, the caudal fin structure may not be depended upon as a taxonomic feature. However, this fin need not be totally ignored in this connection, and it is likely that it may be useful in minor classification; for example, in the *Perciformes*, the *Serranidæ* possess a comparatively lowly specialised homocercal caudal, while the

Labridæ are provided with a highly specialised form, and this corresponds very well with specialised features in general.

4. *Gephyrocercy.*—Something must be said concerning the gephyrocercal caudal fin, since the tendency, unfortunately, has been to depart from the true definition of the term. Gephyrocercy is synonymous with secondary diphycercy; to be gephyrocercal, the tail must have lost its original caudal fin, and the secondary fin have been formed by the union, round the abbreviated extremity, of the rays of the dorsal and ventral median fins; moreover, it must be perfectly symmetrical externally and internally, so that the dorsal and ventral rays are equal in number.

Such a caudal fin has been observed in two forms only among the Teleostei, viz., *Fierasfer* and *Orthagoriscus*, as Ryder has pointed out.

Dollo* gives gephyrocercy a very wide application, attributing it to most forms which have a continuous median fin, such as the eels; but I believe that all these forms can be shown to possess homocercal caudal fins. Several authors have followed Dollo's statements, and some mis-statements will require correction; for example, *Mastacembelus* is usually considered to possess a gephyrocercal caudal fin, whereas its internal structure is as typically homocercal as could be desired.

As yet, I have not been able to study the caudal fin of the Dipnoi in detail, but it is highly probable that this group will be found to afford the best examples of the gephyrocercal form.

There are many other terms which are sometimes used in connection with caudal fins, but I believe that the four terms here dealt with, viz., protocercal, heterocercal, homocercal, and gephyrocercal will include any type of piscine caudal fin likely to be met with.

III.—*Morphology of Parts.*

A word will be necessary on the morphology of the various parts of the endoskeleton. Opinions are undivided on the nature of the urostyle; this structure is the result of the fusion of several vertebræ which occupied the upturned part of the vertebral axis.

After a careful examination of the caudal fins of a large number of Teleosts, and also of some Elasmobranchs and Ganoids, I have come to the conclusion that there is very considerable evidence that the hypurals of the fin are, in general, the result of the fusion of radials or somactids with hæmal arches; apart from actual evidences, I hope to bring forward in the early future strong theoretical reasons why this should be so. There is reason for supposing that the caudal fin was supported primarily in a manner similar to that of other median fins, and that the present hypaxial supports are only a modification of this primary form. Modification was inevitable owing to the adoption of a propulsive function by the tail, and fusion of parts was adopted to ensure the requisite strength. In a large number of caudal fins, the line of fusion can still be traced in the adult. The same thing applies to the formation of epurals; they consist of neural arches fused with radials or somactids.

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* "Sur la phylogénie des dipneustes," 'Bulletin de la Société Belge de Géologie,' etc., vol. 9, p. 93, 1895.

It should, however, be remarked that it does not necessarily follow that every hypural and epural is a compound structure, since, if necessary, there is no reason why a hæmal arch or a neural arch should not itself support fin-rays; in fact, this frequently appears to be the case, but only in connection with the small rays at the anterior end of the fin.

However, radials do not always fuse with neighbouring parts of the skeleton of the caudal fin, but frequently remain free; such is more usually the case dorsally, though radials commonly remain as such ventrally, too. This point needs to be emphasised, for it is sometimes assumed that radials or somactids do not share the support of the caudal fin-rays—at any rate ventrally.

Unfortunately, the species chosen for this paper do not illustrate this hypaxially, but *Clupea* and *Trigla* possess radials dorsally. The persistence of dorsal radials is interesting as illustrating that, under adverse conditions, such as overcrowding, due to excessive upturning of the end of the axis—the radials have persisted while the corresponding neural arches have disappeared or have been greatly reduced.

In conclusion, a detailed study of the piscine caudal fin has forced me to support the theory that the present homocercal caudal is, in reality, a posterior anal, which owes its present position to the great abbreviation of the axis, coupled with the excessive upturning of the end of the chorda. I will even go so far as to suggest that the heterocercal Elasmobranchs may come to be considered as affording evidence for this view, and I hope before long to present all my reasons for consideration.

Some Experiments with the Venom of Causus rhombeatus.

By H. E. Arbuckle, M.B., West African Medical Service.

(Communicated by A. Alcock, C.I.E., F.R.S. Received November 5,—Read December 9, 1909.)

In Sierra Leone there are numbers of a small viperine snake of the genus *Causus*, known locally as "chicken snakes," of which it has been my good fortune to get several fine specimens. The largest was 463 mm. long, the tail being only 35 mm.

The poison-gland is remarkably long, practically one-fourth the length of the snake. It lies dorsally, immediately beneath the skin, and shows no indication of attachment to any other structure. It is flat and ribbon-like, with a maximum breadth of about 6 mm. It is of a greyish-pink colour, spotted in its middle third. It stretches as far back as the posterior level of the heart. At the quadrato-mandibular joint it narrows into a flat duct which becomes attached to underlying structures and is covered by fascia. The duct ends in an ampulla which just touches the maxilla when the fang is erected. The entire gland can be seen by merely reflecting the skin.

The venom seems to be ejected by the contraction of the gland itself. In a chloroformed snake, with the gland exposed by reflection of the skin, a slight pinch of the gland with the forceps causes a vigorous contraction. If the end of the gland be pinched, a peristaltic contraction forwards takes place, towards the duct, and some venom is ejected. If the gland be squeezed in the middle, a peristaltic contraction forwards occurs on both sides of the forceps.

In the experiments here recorded the venom was used in three ways—fresh venom dissolved in sterile salt solution, dried venom dissolved in sterile salt solution, and the alcoholic precipitate of venom re-dissolved in sterile salt solution.

The action of the venom, which seemed the same in all three methods of administration, was shown in local ecchymosis, and in drowsiness and muscular weakness. In about half the cases there was marked slowing of the respiration with great difficulty in inspiration. *Post-mortem* examination showed in most cases superficial hæmorrhage into the viscera, especially the heart and lungs, the lungs being in two cases filled with fluid and deeply discoloured by hæmorrhage.

Experiment 1.—After killing the snake the two glands were at once removed and squeezed into sterile salt solution in a hypodermic syringe, and the whole was injected into the pectoral muscles of a chicken weighing 5 ounces. 10.20 a.m., venom injected. 10.27, chicken looked sleepy, stood without moving, respiration 47. 10.55, animal propped itself up against the side of the box, respiration 40. 11.15, respiration 35, inspiration difficult, ecchymosis over abdomen and lower part of thorax. 11.45 a.m. to 2 p.m., respiration gradually slowed from 35 to 24. 2.45, the animal when placed flat on its back remained in that position with quickened respiration. 3.15, died quietly. *Post mortem:* a large, dark maroon-coloured ecchymosis over the abdomen, most of the thorax, and the root of the neck: the pectoral muscles of the side injected were deeply blood-stained. On opening the body-cavity superficial hæmorrhages were found in the gizzard, liver, and heart: the right auricle contained a small, dark, soft clot; the endocardium of the right ventricle was much congested; the lungs were pale, with no excess of fluid.

Experiment 2.—The venom, mixed with sterile salt solution, of *one* gland was injected into the pectoral muscle of a chicken weighing 8 ounces, at 10 a.m. 10.5, wings began to droop, chicken gasped and kept swallowing as if there were some obstruction in the throat, respiration 22, inspiration very difficult. 10.40, respiration 16, inspiration much prolonged. 11.40, respiration 16, chicken could not get up when placed on its side. 11.42, respiration 11. 11.45, convulsions, with emission of blood-stained froth from the mouth, death. *Post mortem*: the vessels in the neighbourhood of the puncture were much enlarged, as were those of the abdominal wall and peritoneum: the iliac veins and vena cava were engorged with dark blood: a few hæmorrhages were found in the right lung: the left lung was of a dark cherry colour and full of frothy fluid: the right auricle and ventricle contained each a dark soft clot. In this case the comparatively rapid death was probably due to some of the poison having entered a blood-vessel; for, after the injection, some clotted blood was found in the needle of the syringe.

Experiment 3.—The venom of one gland was mixed with sterile salt solution in a syringe, and half of the mixture was injected into the left pectoral muscle of a fowl weighing 17 ounces, at 10 a.m., the respiration then being 30. 10.30, respiration 24, a little irregular; fowl squatting. 1.30 p.m., fowl quite lively. 10 a.m. on following day, dark ecchymosis near puncture; vessels on both sides of thorax and abdomen, but particularly of left side, much enlarged. 7 a.m. on third day, skin of whole abdomen green; the left leg of the fowl stiff. The fowl completely recovered by the end of the third day.

Experiment 4.—The venom of both glands was dried in the sun and its weight was found to be 9 centigrammes. It was then dissolved in 21 minims of sterile salt solution, and 14 minims of the solution (= 6 centigrammes of the venom) was injected into one chicken (A), and the remainder (= 3 centigrammes of venom) into another chicken (B), at 10.15 a.m., the injection in each case being made into the right leg. 10.55 : "A" respiration 29 ; "B" respiration 33, and sits with right wing outstretched ; both fowls allow themselves to be touched without attempting to move. 11.35 : "A" right leg dark blue and swollen ; "B" right leg slightly ecchymosed, superficial vessels enlarged. 1.30 p.m. : "A" respiration 34, abdomen ecchymosed and superficial vessels enlarged ; "B" respiration 35, some of the superficial abdominal vessels dilated, and slight ecchymosis of abdomen. 4.30 p.m. ; both fowls lively and eager to feed : in "A" the leg is swollen and the ecchymosis has spread from the abdomen to the thorax ; in "B" the leg is swollen and only the abdomen is ecchymosed. 6.30 a.m. on second day : "A" right side of abdomen and thorax swollen and discoloured ; "B" whole of abdomen and right side of thorax ecchymosed. 6.30 a.m. on third day : both fowls limp, but are quite lively. 10 a.m. on third day : "A" quite lively, but its abdomen is still discoloured ; "B" is almost normal, but has very slight ecchymosis at site of puncture. 10 a.m. on fourth day, both fowls are quite recovered.

Experiment 5.—The venom of both glands was dried in the sun, and found to weigh 16 centigrammes. 9.15 a.m., 12 centigrammes of the venom was dissolved in sterile salt solution and injected into the right pectoral muscle of a fowl. 10.30, respiration 36 ; ecchymosis all over right side of thorax and abdomen. 11.30, respiration 33, ecchymosis spreading to left side of thorax. 12.30, respiration 28. 2.15 p.m., respiration 28, ecchymosis spread over left side of abdomen and thorax and right wing. 2.40, convulsions, fowl died. *Post mortem* : subcutaneous tissue of abdomen and thorax full of blood-stained fluid ; right pectoral muscle flabby and discoloured with blood ; peritoneal vessels dilated, peritoneal cavity contains blood-stained fluid ; tiny hæmorrhages in pericardium, vessels of myocardium dilated ; both lungs were hæmorrhagic and contained much dark-coloured frothy fluid.

Experiment 6.—Two glands were cut up and treated with alcohol (90 per cent.). Two days afterwards the alcohol was filtered off, and the precipitate was redissolved in sterile salt solution. At 4.45 p.m. the whole of the solution was injected into the pectoral muscle of a fowl. 4.48, fowl staggered and fell, had slight convulsions and then lay quiet. 4.57, fowl staggered to rise, but failed ; respiration 24. 5.10 fowl struggled to its feet ; very drowsy, eyes closed, wings drooping ; respiration 19. 5.22, fowl gasped, respiration 14, inspiration difficult. 5.31, respiration 18, noisy, as if the air passages contained fluid. 10.0, fowl very unsteady, comparatively cold; abdomen ecchymosed. Fowl died during the night. *Post-mortem* examination showed ecchymosis of skin of abdomen and thorax, and effusion of blood into the pectoral muscle at the site of the puncture; the lungs were bright red and contained much blood-stained fluid.

On the Comparative Action of Stovaine and Cocaine as measured by their Direct Effect upon the Contractility of Isolated Muscle.

By V. H. Veley, F.R.S., and A. D. Waller, F.R.S.

(Received November 8,—Read December 9, 1909.)

It has been shown by Veley* that the affinity values of stovaine and cocaine by the methyl orange and borax precipitation methods are approximately equal. The method of measuring physiological activity of anæsthetic drugs, as described by Waller,† affords an independent control that can usefully be compared with affinity values.

Stovaine, sometimes called amyleine hydrochloride and originally prepared by Fourneau, has been successfully applied in recent years, especially for spinal anæsthesia, as also for general surgery and dentistry. As it is used for the same purposes and in doses of the same order as cocaine, the relative value of the two drugs has been compared in a series of memoirs or notices, more than 150 in number, in various clinical journals. The general conclusions arrived at are: (1) as anæsthetics the drugs are of equal value, but stovaine produces vaso-dilatation, cocaine vaso-constriction; and (2) stovaine is less toxic than cocaine. The statement has even been made that stovaine does not produce any toxic effect.

Though, as stated above, the affinity values are approximately equal, yet the chemical constitution of the two compounds is wholly dissimilar. Stovaine is the hydrochloride of methyl ethyl dimethylamino-methyl carbinol benzoate $HClC(CH_3)[CH_2N(CH_3)_2](C_2H_5)OBz$ and the base (molecular weight = 235), a benzoyl derivative of a diamino-tertiary amyl alcohol $C(CH_3)_2(C_2H_5)OH$ (Fourneau).‡

Cocaine (molecular weight of base = 303) is the methyl ester of benzoyl ecgonine and derived from tropine by the replacement of the hydroxylic hydrogen by the benzoyl group, and of hydrogen by the methylated carboxylic group (Willstaetter*), thus:—

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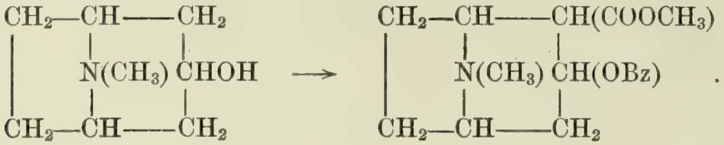
* 'Trans. Chem. Soc.,' 1909, p. 763.

† 'Proc. Roy. Soc.,' June 24, 1909.

‡ 'Comptes Rendus,' 1904, vol. 138, p. 766.



Tropine. Cocaine.

If stovaine and cocaine had not been used for identical purposes in surgery, no comparison would ever have been instituted in chemistry.

The crystalline samples of stovaine, kindly supplied by Messieurs Poulenc Frères, and of cocaine hydrochloride, by Messrs. Burroughs Wellcome & Co., had been tested and used for experiments on the affinity values by the methyl orange and borax precipitation methods. The solutions were made upon the same day of *n*/10 concentration, and subsequently diluted to *n*/500 and *n*/1000.

Simultaneous records were taken with the same pair of muscles as a "crucial" experiment.

		Percentage calculated as base.
n/500	stovaine	0·0470
n/1000	„	0·0235
n/500	cocaine hydrochloride	0·0610
n/1000	„ „	0·0305

From the records (*n*/1000 concentration, fig. 1, line 1) abolition took place in the following times: stovaine 22, cocaine 22·5 minutes, and the recovery in saline (at "sal.," end of 1st, 2nd, and commencement of 3rd lines) was rather better in the case of cocaine than in that of stovaine.

On reversing the solutions it was found that too long a time would be required for total abolition, so solutions of double the strength were substituted.

The times required for abolition were then—stovaine 4 minutes and cocaine 5·5 minutes, and the subsequent recovery was rather better for the latter than for the former. It is evident that, judged by this method, there is no appreciable difference between these two drugs; the slight difference observed is rather in favour of cocaine being the less toxic.

Comparative experiments on nerve with solutions of stovaine and cocaine of the same molecular concentration also showed quite a trifling difference.

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* 'Ber.,' 1891, vol. 34, p. 3108.

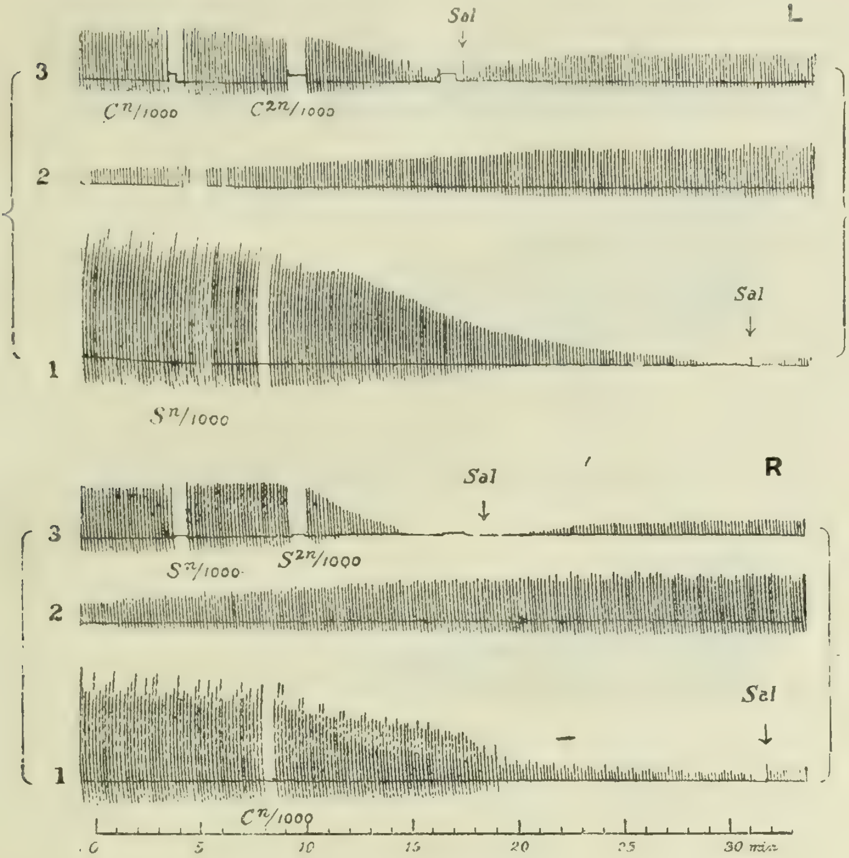

FIG. 1.—Stovaine and Cocaine.

Simultaneous record of two sartorius muscles, R and L; the former in cocaine *n*/1000, then in saline, then in stovaine *n*/1000 and *n*/500, finally in saline; the latter in stovaine *n*/1000, then in saline, then in cocaine *n*/1000 and *n*/500, finally in saline.—June 9, 1909. (The records read from left to right.)

The methyl analogue of stovaine or trimethyl dimethylamino-methyl carbinol benzoate $HClC(CH_3)_3 CH_2N(CH_3)_2.OBz$, derived from the tertiary butyl alcohol $C(CH_3)_3OH$, was also studied.

The sample, also supplied by Messieurs Poulenc Frères, had previously been found to have an affinity value slightly above that of stovaine.

In this case simultaneous records were made with a pair of muscles with solutions of different concentrations, namely:—

	Percentage calculated as base.
n/500 methyl stovaine	0·050
n/1000 " "	0·025

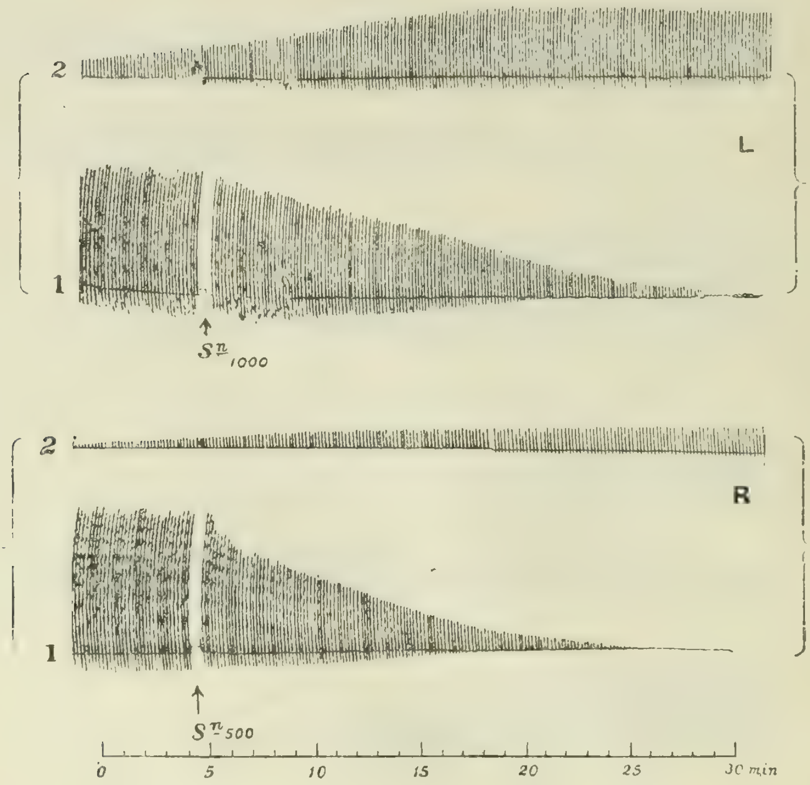

Fig. 2.—**Methyl Stovaine.**

Simultaneous record of two sartorius muscles, R and L, the former in $n/500$, the latter in $n/1000$ solution of methyl stovaine (in saline).

As appears from these records, abolition took place in the following times: 23·5 minutes for $n/1000$, and 18 minutes for $n/500$; the recovery in the former case was nearly complete, in the latter very incomplete.

It appears that, as regards the $n/1000$ solution, methyl stovaine does not differ materially from stovaine, the times of abolition and degree of recovery being almost identical. As regards the $n/500$ solution, though the time required for abolition is rather longer than might be expected, the degree of recovery is quite in accord with that of stovaine.

The general conclusion is, therefore, that the substitution of the ethyl group in stovaine by the methyl group produces no alteration in toxic effect, and this result is quite in accordance with the affinity values of the two substances in question.

The physiological activity of these drugs as compared with that of quinine, to which we have paid special attention, is as follows :—

Quinine	100
Stovaine	40
Methyl stovaine	40
Cocaine	40

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Page

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ON THE REFRACTION AND DISPERSION OF AIR, OXYGEN, NITROGEN, AND HYDROGEN, AND THEIR RELATIONS. By Clive Cuthbertson and Maude Cuthbertson 151

ON THE REFRACTION AND DISPERSION OF SULPHUR DIOXIDE AND HYDROGEN SULPHIDE, AND THEIR RELATION TO THOSE OF THEIR CONSTITUENTS. By Clive Cuthbertson and Maude Cuthbertson 171

ON THE NATURE OF THE HYDROGEN FLOCCULI AND THEIR STRUCTURE AT DIFFERENT LEVELS IN THE SOLAR ATMOSPHERE. By George E. Hale, For. Mem. R.S., and Ferdinand Ellerman. (Plate 1) 177

ON THE DISTRIBUTION OF THE RÖNTGEN RAYS FROM A FOCUS BULB. By G. W. C. Kaye, B.A. (Cantab.), D.Sc. (Lond.), A.R.C.Sc. 189

ON THE NATURE OF THE IONISATION OF A MOLECULE BY AN α-PARTICLE. By R. D. Kleeman, D.Sc., B.A. 195

THE CRYSTALLINE STRUCTURE OF IRON AT HIGH TEMPERATURES. By Walter Rosenhain, B.A., D.Sc., and J. C. W. Humfrey, B.A., M.Sc. (Plate 2) 200

THE SPECTRUM OF MAGNESIUM HYDRIDE. By Prof. A. Fowler, A.R.C.S., F.R.A.S. (Abstract) 210

THE RELATION OF THALLIUM TO THE ALKALI METALS: A STUDY OF THALLIUM-ZINC SULPHATE AND SELENATE. By A. E. H. Tutton, M.A., D.Sc., F.R.S. .. 211

A CRITICAL STUDY OF SPECTRAL SERIES. PART I.—THE ALKALIES, H AND He. By W. M. Hicks, F.R.S. (Abstract) 226

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CONTENTS.

Page

Address of the President, Sir ARCHIBALD GEIKIE, K.C.B., at the Anniversary Meeting on November 30, 1909 151

The Velocity of Reaction in the "Absorption" of Specific Agglutinins by Bacteria, and in the "Adsorption" of Agglutinins, Trypsin, and Sulphuric Acid by Animal Charcoal. By GEORGES DREYER, M.A., M.D., Professor of Pathology in the University of Oxford, and J. SHOLTO C. DOUGLAS, M.A., B.M., Philip Walker Student in Pathology in the University of Oxford, formerly Radcliffe Travelling Fellow . . . 168

On the Absorption of Agglutinin by Bacteria and the Application of Physico-chemical Laws thereto. By GEORGES DREYER, M.A., M.D., Professor of Pathology in the University of Oxford, and J. SHOLTO C. DOUGLAS, M.A., B.M., Philip Walker Student in Pathology in the University of Oxford, formerly Radcliffe Travelling Fellow 185

Observations on the Rate of Action of Drugs (Alcohol, Chloroform, Quinine, Aconitine) upon Muscle as a Function of Temperature. By V. H. VELEY, F.R.S., and A. D. WALLER, M.D., F.R.S. 205

An Examination of the Physical and Physiological Properties of Tetrachlorethane and Trichlorethylene. By V. H. VELEY, F.R.S. 217

The Photochemical Formation of Formaldehyde in Green Plants. By S. B. SCHRYVER, D.Sc., Ph.D., Lecturer on Physiological Chemistry in University College, London 226

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Three Shillings.

No. B 554.

February 3, 1910.

NOTICE TO AUTHORS AND COMMUNICATORS.

The Council has had under consideration the rapid increase of the Society's expenditure on publications. In view of the necessity for economy, authors of papers are urgently requested to see that their communications are put in as concise a form as possible. Delay in decisions regarding publication, as well as subsequent trouble to authors, is often caused by diffuseness or prolixity. MSS. must be type-written or at least written in a legible hand, and properly prepared as copy for press. Type-written transcript should in all cases be carefully revised by the author before being presented. It is desirable that authors should retain copies of their MSS. for reference.

Authors are further requested to send in all drawings, diagrams or other illustrations in a state suitable for direct photographic reproduction. They should be drawn on a large scale in Indian ink on a smooth white surface, with lettering adapted to a reduction in scale. Great care should be exercised in selecting only those that are essential. Where the illustrations are numerous, much time would be saved if the authors would indicate in advance those which, if a reduction of their number is found to be required, might be omitted with least inconvenience.

" It shall be the duty of each Fellow or Foreign Member to satisfy himself that any letter, report or other paper which he may communicate, is suitable to be read before the Society."—Statute VI, Cap. xii.

NOTICES TO FELLOWS OF THE ROYAL SOCIETY.

The Council have directed that the Minutes of the Meetings of the Society shall be sent out as an inset in the 'Proceedings,' separately paged, and shall afterwards be republished in the 'Year-Book.'

The 'Proceedings,' both the Physical and the Biological Series, are sent in the ordinary course by post to every Fellow of the Society who resides within the limits of the Postal Union. On application to Messrs. Harrison and Sons, 45, St. Martin's Lane, these will be bound in volumes, in cloth, for 2s. 6d., or the cases for binding may be purchased, price 1s. 6d.

The 'Philosophical Transactions' are delivered, in volumes, in cloth covers, only to those Fellows who call for them, or who send a written application to the Assistant Secretary. Such an application may, if so desired, be filed as a standing order.

The 'Philosophical Transactions' are also delivered in the form of separate Papers, post free, immediately on publication, to those Fellows who desire to have them in that form. And, on application to Messrs. Harrison and Sons, 45, St. Martin's Lane, these will be bound in a cloth case for 2s. 6d., or the cloth cases for binding may be purchased, price 1s. 6d.

SUBSCRIPTION TO THE PUBLICATIONS OF THE ROYAL SOCIETY.

The 'Proceedings' are on sale to the public in numbers, on publication, at the prices appearing on the wrappers; or as completed volumes, bound in cloth, Series A, 20s., Series B, 25s. per volume.

The 'Proceedings' may also be obtained by subscription, for either or both series, paid in advance, at special rates as under :—

Series A, 15s. per volume.
Series B, 20s. per volume.

For this advance subscription, each series may be had in separate numbers, immediately on publication, or as completed volumes, bound in blue cloth.

The physiological activity of these drugs as compared with that of quinine, to which we have paid special attention, is as follows:—

Quinine	100
Stovaine	40
Methyl stovaine	40
Cocaine	40

Address of the President, Sir Archibald Geikie, K.C.B., at the Anniversary Meeting on November 30, 1909.

Since our last Anniversary, death has removed from the Society's membership a number of distinguished names.

The deceased Fellows are:—

George Gore, died December 23, 1908.
Prof. Harry Govier Seeley, died January 8, 1909.
Francis Elgar, died January 16, 1909.
Wilfrid H. Hudleston, died January 29, 1909.
Sir George King, died February 12, 1909.
Prof. David James Hamilton, died February 19, 1909.
Dr. Arthur Gamgee, died March 29, 1909.
Dr. Gerald Francis Yeo, died May 1, 1909.
Bindon Blood Stoney, died May 5, 1909.
Prof. Daniel John Cunningham, died June 23, 1909.
Prof. Thomas William Bridge, died June 30, 1909.
The Marquis of Ripon, died July 9, 1909.
Rev. William Henry Dallinger, died November 7, 1909.
Dr. William James Russell, died November 14, 1909.

On the Foreign List we have to record with regret the decease of five men of wide reputation:—

Prof. Albert Gaudry, died November 29, 1908.
Prof. Julius Thomsen, died February 13, 1909.
Dr. Georg von Neumayer, died May 24, 1909.
Prof. Simon Newcomb, died July 11, 1909.
Dr. Anton Dohrn, died September 26, 1909.

In addition to the heavy losses which French science has had to deplore within the last two or three years, another has arisen from the decease of Albert Gaudry, who for more than half a century was one of the leaders of palæontological science, not in France alone, but in every country where the history of life upon the globe is studied. By his original investigations at Pikermi, he added a new and vivid chapter to the records of vertebrate existence, while by his volumes on the genetic connections of the animal world, as shown by fossil organic remains, he gave powerful support to the reception of the doctrines of evolution. Great as was his scientific genius, it was not more impressive than the charm of his personal character. Those who were privileged to know him will long mourn an irreparable blank in their circle of friendship.

Astronomical science has lost one of its most notable cultivators, and the United States have been deprived of their most famous man of science by the death of Simon Newcomb. The successive stages in his interesting career have been sketched by himself in his pleasant autobiography. On his frequent visits to this country he never failed to appear at the meetings of the Royal Society, where he was always welcomed. The value of his scientific work was recognised by the Society as far back as 1877, when he was elected a Foreign Member, so that at the time of his death his name stood at the head of our list in point of seniority. In 1890 our highest distinction, the Copley medal, was awarded to him.

The zoological circle of sciences has been deprived of one of its most esteemed Members by the death of Anton Dohrn. The establishment of the great marine biological station at Naples, which he planned and carried out, is one of the most important contributions ever made to the practical development of biological studies, and has given its gifted and enthusiastic founder a high and enduring place in the history of science.

In the modern development of oceanic hydrography no name deserves more honourable mention than that of Georg von Neumayer. His long and enthusiastic devotion to this branch of science and the admirable work done under his direction in the Deutsche Seewarte at Hamburg entitle him to lasting remembrance.

By the death of Julius Thomsen, Denmark has lost one of her most distinguished men of science. At the advanced age of eighty-three he has passed away, leaving an extensive record of experimental researches which had a fundamental part in the foundation of the science of thermochemistry.

Our losses on the Home List amount to fourteen. The late Marquess of Ripon, who became a Fellow of the Society in 1860, filled with distinction many high offices of State. It is on the side of the biological sciences that the losses have chiefly fallen. Anatomy, human and comparative, has not for many years sustained a more grievous blow than that which has come from the untimely death of Daniel John Cunningham. Brilliant as an investigator, luminous as an expositor, and with a singularly attractive personality, he held with distinction for some twenty years the Chair of Anatomy in Trinity College, Dublin. Six years ago he was chosen to succeed Sir William Turner at Edinburgh, where it was fondly believed that he would long continue to uphold and extend the reputation of the famous anatomical school of that University. But he has been cut down in his prime, leaving us the memory of a blameless and genial life, and the example of an untiring devotion crowned with conspicuous success in every branch of investigation, teaching, and administration, to which he applied his remarkable powers.

In David James Hamilton we have lost one of the most experienced pathologists in this country, whose researches on various diseases incident to man and on some of the obscure ailments of sheep have been of lasting benefit. He was elected into the Royal Society only last year. We sincerely regret that he should have been so soon removed from our midst.

Within the last few weeks the death of the Rev. W. H. Dallinger has deprived the Society of one who was widely known and much respected for his microscopic researches into the history of the minuter forms of animal life.

The number of geological Fellows of the Society has been reduced by the loss of two well-known and accomplished men. Wilfrid Hudleston Hudleston belonged to that small but important class, who having no professional ties and possessed of a competence, are enabled to devote themselves to the cultivation of science from pure love and enthusiasm. To his critical skill English geology and palæontology stand under deep obligations. His contributions to our knowledge of the invertebrate fauna of the Jurassic rocks, as well as many thoughtful essays on a wide range of geological and geographical subjects, are valuable additions to scientific literature.

Harry Govier Seeley, trained under Sedgwick at Cambridge, early took up the study of fossil reptiles, which became the main pursuit of his life. His published memoirs on these extinct types gave him a wide reputation, and remain as a lasting memorial of his patient research, critical insight, and suggestive generalisations.

The band of physiologists in our ranks has been thinned by the decease of two of its members. We shall long regret the disappearance of the brilliant, enthusiastic, widely accomplished, and kindly Arthur Gamgee. Those of us who knew Gerald Francis Yeo before he sought the repose of his country home in Devonshire, will cherish the remembrance of his scientific accomplishments, his social charm, and his characteristic Irish humour.

Thomas William Bridge, trained in the Cambridge school of zoologists, was one of the oldest and most respected professors in the Mason College and more recently in the University of Birmingham. His researches on the anatomy of fishes have given him an honoured place among the men who drew their inspiration from Frank Balfour and Alfred Newton.

To systematic botany, and more especially to the botany of India, the late Sir George King rendered important service. His death, at the age of sixty-nine, has removed from our ranks one of the most modest and meritorious of retired Indian officials.

Two of our representatives of engineering science have passed away since this time last year. In Francis Elgar we mourn the loss of one of the most distinguished naval architects of his generation. Bindon Blood Stoney has left many enduring monuments of his skill and resource as a civil engineer in harbour works, bridges, and other constructions over the face of Ireland, while his treatise on the Theory of Stresses has long held high rank in engineering literature.

George Gore, who died at the ripe age of eighty-three, will be remembered for the number and value of his contributions to chemistry and electro-metallurgy. His memory will also be kept alive in the Royal Society by a bequest which he has left to us. Only a few days ago our losses for the year have been increased by the death of the able chemist, William James Russell, who has recently been a frequent contributor to our 'Proceedings,' and whose kindly face for more than thirty years has been familiar at our meetings.

In considering the special subject to which this Address should be devoted, I have been led to select one on which I believe much misapprehension to exist, not only in the world outside these walls, but even among our own Fellows. I have often been impressed by the exceedingly imperfect or erroneous conception generally entertained as to the work in which the Royal Society is engaged. We are known to hold weekly meetings for the reading and discussion of scientific papers, and to publish these papers in the long series of volumes wherein the record of our activity is preserved. But it is not commonly known that these meetings and the publications to which they give rise, though they constitute the most important part of the labours of the Society, so far as relates to the progress of discovery, form only a portion of a programme which is every year becoming fuller and demanding more time, thought, and funds for its accomplishment. I have therefore judged that it might serve a useful purpose were I on this occasion to offer a brief outline of the various directions in which the energies of the Society are employed, in the hope that when some of the difficulties which confront us become more widely known, means may be found for adequately coping with them.

Ever since its foundation nearly two centuries and a half ago, the main occupation of the Royal Society has been the holding of meetings for the reading and discussion of contributions to natural knowledge, and the publication of these papers, or abstracts of them, in our printed records. The 'Philosophical Transactions,' dating back to the Society's infancy, form a series of volumes of which we may well be proud, for it is a chronicle not merely of the doings of the Society, but of the onward march of science in every branch of its domain. In the course of time, however, the conditions in which the progress of investigation and discovery advances have greatly changed. When the Royal Society was founded it was the only learned body in this country specially devoted to the prosecution of scientific enquiry, and such it continued to be for generations. But the rapid growth of science during the last century has shown that no single Society can now serve to supply the needs of the whole vast field of investigation in every department of nature. Most of these departments, one after the other, have had special societies created for their exclusive cultivation, each of which records the progress of research in its own territory. At first the Royal Society, long accustomed to reign with undisputed sway over the whole realm of natural knowledge, was disposed to look with disfavour on this multiplication of separate and independent institutions. But that time has long since passed away. Subdivision is now admitted to be necessary and, if properly directed, even desirable. Hence this Society, like a proud parent, now rejoices in the growth and energy of the increasing family which has grown up around her, while she in turn is regarded with respect and esteem by the various members of that family, among whom there is a general desire to be enrolled in her ranks.

Nevertheless it is impossible not to perceive that the rise of all these younger societies has materially affected the position of the Royal Society in regard to the general advance of modern science. This society is no longer the general depository of the records of that progress in all its branches. So completely, for instance, do the Geological and Chemical Societies provide for the requirements of their respective fields of investigation, that communications from these fields come now comparatively seldom before us. If one desires to follow the modern growth of geology or chemistry, one must turn for its record to the publications not of the Royal Society, but of the two learned bodies that are specially devoted to the cultivation of these sciences. Nor can we see any reason why this process of devolution should not continue in the future. Hence if the system of reading and publishing papers which has been in use here for so many generations is to be perpetuated without modification, there may come a day when every great department of natural knowledge will be provided with its own special society, and then we may ask in anticipation, what will be left for the meetings of the Royal Society. For myself I do not believe that such a time of impoverishment ever will befall us. We cannot, and would not if we could, do anything to prevent the foundation of fresh societies for sciences that have not yet been provided with them. But we may so adjust our programme as to bring it into harmony with modern conditions, and thus to maintain and extend the prestige and usefulness of the Royal Society. The danger to which I have referred, however, is by no means imaginary, and it should be faced before it has time to become serious.

Notwithstanding the changes brought about by the multiplication of scientific societies during the last hundred years, the reading of papers and the printing and publication of them still remains the most prominent function of our Society. The 'Philosophical Transactions' and 'Proceedings' continue to appear, and to maintain their high reputation. Even although their range of subjects has been lessened by the appearance of the many other scientific serials now published, they still form one of the most outstanding chronicles of the progress of research. With the view of increasing their usefulness, the Society some years ago departed from the time-honoured practice of dealing with natural knowledge as one great subject, and now groups its papers in two separate series, one devoted to Physical (A) and the other to Biological (B) questions. It is undoubtedly a considerable convenience to have the memoirs in each of those two great divisions gathered together into a separate series of volumes. More recently the practice has grown up of introducing a similar principle in the grouping of papers to be read at the weekly meetings of the Society. It was hoped that by taking the physical papers on one day and the biological communications on another a better attendance could be secured, especially of the representatives of each division. I cannot say that this arrangement has been attended with the success which was anticipated. It has, however, its humorous side, as may be seen on most Thursday afternoons. The preliminary half hour of general intercourse which is afforded in the tea-room undoubtedly forms one of the most useful and most generally appreciated features in the programme of the Society's functions. It provides convenient opportunities for the cultivation of personal relations between the workers in all branches of research, and much valuable service is undoubtedly done by the conversation and informal friendly discussions which then take place. But when, at the end of the brief half hour, the ordinary weekly meeting of the Society opens in the adjoining room, a singular contrast is presented between the two companies. The tea-room continues to be a scene of animated talk long after the bell has announced the commencement of the meeting. If the papers to be read are biological, some of the biologists will be found to adjourn with the President and officers into the meeting room to listen to the reading of these papers. But the physicists remain for the most part outside. In like manner, on a day set apart for physical questions, the biologists will show a similar predilection for prolonging the amenities of the tea-table instead of hearing the papers read. In either case, each time that the door between the two rooms is opened the loud hum of conversation bursts into the meeting with a volume which for a few moments may make the speaker inaudible.

That there are some practical advantages in this separation of subjects cannot be gainsaid, and I would not for a moment seek to undervalue them. But I confess I am often led to consider this subject with feelings of regret and misgiving, and to ask myself whether the conveniences afforded by the subdivision are not more than compensated by the disadvantages that accompany them. Undoubtedly, the constantly quickening pace of the march of science makes it every year increasingly difficult for those whose lives are devoted to the active and engrossing prosecution of research in one special department of enquiry to keep in touch even with the broader features of the advance that is being made in other departments. We cannot be surprised that a man whose whole energies are absorbed in one line of study should neither care to listen to, nor to burden his library shelves with, papers in other lines, full of technicalities which he has had no time to master, and written therefore in a language which to him is more or less unintelligible. In this way the workers in widely separated fields of enquiry tend to be more and more completely isolated from each other.

But surely such isolation is a defect in our organisation which deserves serious attention. It cannot be for the general good of scientific progress that the eyes of an investigator should seldom or never be lifted from his own field of work, nor his ears be open to the reports of the advances made in other fields that lie outside of his immediate interests. The wider his outlook, the greater must obviously be his capacity for judging of the general bearings of discovery in his own domain on other departments of research, and the broader and more intelligent will be his sympathies with the whole range of activity on which the continued march of natural knowledge depends.

The Royal Society is still the one great institution in this country which draws its members from the cultivators of every branch of science, and which freely opens its publications to receive their communications of observation and discovery. It should thus be specially fitted to bring the workers on the two sides of science, physical and biological, into touch with each other. It has recognised and in various ways endeavoured to discharge its duty in this respect. In its Croonian and Bakerian Lectures it has given to the world many masterly expositions of the progress of research in different branches of enquiry. It has likewise provided, by one of its standing orders, for occasional meetings devoted to the discussion of papers of general interest specially prepared for the purpose. Nevertheless, it may be urged that some more frequent and effective procedure might still be devised to lessen the evils of isolation and to make the work that is in progress in one section of the scientific domain more comprehensible in the others. It is futile to find fault with the technicalities of a science. These are its symbols and language with which its students cannot dispense. But without trying to provide for all the needs of the "man in the street," it is often possible to give the gist of an observation or a discovery in simple words that will convey a definite conception of what has been observed or discovered. And thus a subject which, when expounded in brief technical phraseology, repels men of another science, may yet be made interesting and suggestive to these same men.

It may be worthy of consideration whether in those branches of science which, having special societies of their own, are seldom represented by papers at our meetings or in our publications, some of their cultivators might not be invited from time to time to bring before the Society reports of recent advances in their different fields of research. Would it not be practicable, for example, to find among the many distinguished chemists in our ranks a few who would be willing to present occasionally at our meetings, in language intelligible to a general audience of scientific men, an outline of the latest progress, present condition, and future problems of some section of their great science?

But above all there is an aspect of scientific thought, which although fully recognised by the early fathers of the Royal Society, is too apt to be overlooked amidst the engrossing pressure of modern research. I allude to the philosophy of science. At intervals in the progress of scientific enquiry it is desirable to look at the subject from the philosophical side, and to seek for a correlation and synthesis of the various processes of nature which discovery has revealed. The mental vision required for this quest is not given to more than a few gifted minds. But we can count among the number of our Fellows more than one admirably qualified by wide knowledge and rare powers of generalisation to present a connected view of the broader bearings of discovery in the scientific domain in which each is a master. Memoirs of this type will, I trust, continue to be laid before us, perhaps at more frequent intervals, thus upholding the renown of our 'Philosophical Transactions' and sustaining the prestige of the Society.

Before passing from the subject of our publications, there is one aspect of them to which I should like to refer—the cost of their production. This item of our expenditure has increased so much in recent years as sometimes to raise serious doubts whether we shall continue to be able to defray it out of our annual income. A large part of the outlay lies in the cost of the illustrations. There cannot, of course, be any difference of opinion as to the importance and necessity of the adequate illustration of the papers published by the Society. But while this general admission is made, it may be fittingly qualified by the statement that many authors are somewhat prone to over-illustrate their papers. Every care is taken by the Officers, the Referees, the Sectional Committees, and the Council as far as possible to keep this source of expenditure within reasonable bounds, and, as might be anticipated, their efforts in this matter are not always appreciated by the authors. Even in the case of the most important memoirs the unenviable duty is sometimes imposed on the Secretaries of asking the writers to undertake the painful task of deciding which of the illustrations, whereon much care had doubtless been bestowed, could be eliminated with least detriment to the text, so as to bring the total cost within the limits of the Society's means. It would obviously be more agreeable to all concerned to reproduce every figure and plate of a worthy, if expensive, memoir, and to do so in the most excellent style of modern art. Were it not, however, for the annual grant of £1000 voted by Parliament to assist the Society in its publications, we should not be in a position to deal as liberally as is now done in the matter of illustration. Yet it should be remembered that the Royal Society does not appropriate the whole of that grant to its own uses, but considers the claims made upon it by other learned bodies. Until, therefore, some generous donor shall provide the necessary addition to our resources, I am afraid that we must continue our present vigilant watch over the expenses of our publications, while securing, it is hoped, that nothing absolutely essential is held back.

Besides the issue of the 'Philosophical Transactions' and 'Proceedings,' the Royal Society has been engaged for more than forty years in the preparation and publication of a 'Catalogue of Scientific Papers' from the beginning to the end of the nineteenth century. Of this great work twelve volumes have been published, bringing it down to the year 1883. The materials for the remaining seventeen years are now in course of compilation, but so great has been the increase of scientific serials during the latter portion of last century that the number of entries for these years will probably equal, if it does not exceed, that for the whole period from 1800 to 1883. While the laborious task of preparing this Authors' catalogue is in progress, under the able direction of Dr. McLeod, the preparation of an Index of Subjects is being simultaneously carried on. One volume of this Index, devoted to Pure Mathematics, has been issued. A second volume, dealing with Mechanics, will soon be ready for publication, while those for Physics and Chemistry are far advanced. The whole Index, when completed, will consist of seventeen volumes, grouped according to the scheme of the 'International Catalogue of Scientific Literature.' The supreme importance of such a Catalogue of Scientific Papers and Index of Subjects for all engaged in active scientific life has everywhere been recognised. But the cost of producing such works has been a severe burden on the resources of the Royal Society, and, indeed, could hardly have been continued, but for the generous aid afforded by our colleague, Dr. Ludwig Mond, who has taken so keen an interest in the progress of the compilation. The task of carrying on the Catalogue from the beginning of the present century was felt to be beyond the power of this Society alone. But the project has not been allowed to lapse, for it has now become an international undertaking to which the Governments of various countries contribute. The central office remains in London, while the Royal Society has had to continue its financial responsibility for the printing contracts.

But over and above the preparation and issue of what are more properly its own serials, the Society from time to time undertakes the charge of other extraneous publications. Such, for instance, at the present time, are the volumes embodying the physical results of the National Antarctic Expedition in the "Discovery" during the years 1901—1904, of which four volumes have been issued, and the last, dealing with the Meteorology, is now in preparation. Valuable Reports drawn up by Fellows in past years have in this way been published by the Society, of which the volumes on Krakatoa, Funafuti, the Ceylon Pearl Fisheries, and the Magnetic Survey of South Africa may be cited as examples.

From what I have now said it will be obvious that had the Royal Society no other duties to discharge save those in connection with the preparation of its publications, it would, like other scientific societies, have work enough on hand fully to occupy its time and absorb its resources. But the performance of these duties fills up only part of its programme. In this respect the Society differs from other learned bodies. It possesses a large and diversified field of activities about which most even of our Fellows know little, and the world outside still less. Our Year Book, indeed, presents a formidable list of the public functions which have devolved upon the Royal Society. That list, however, conveys no adequate idea of the varied and even exacting character of some of its items. But over and above the functions therein enumerated, others of a less public kind make large demands upon the time and thought of many of our Fellows.

In the first instance the general business of the Society is arranged by the President and Officers, so that all matters of importance are placed before the Council, which considers and decides them. As far as its limited numbers will admit, the Council is so chosen as generally to secure that among its members there shall be one or more representatives of each great division of science and of all parts of the country. But it would be wholly impossible for the Council to enter into the multifarious details of the wide range of matters which are under its control. Hence it has been necessary to delegate the consideration of these details to Committees selected from among the Fellows most competent to deal with them. It may give some idea of the extent of work to be done and the subdivision of labour that has been found necessary, to state that at present the Standing Committees and Boards exceed thirty in number, and that, in addition, Special Committees are constantly being appointed for the consideration of questions that from time to time arise. The deliberations of the Committees are reported to the Council for its information or guidance.

For many years past the Royal Society has acted as a kind of board of advice to the Government of the country in matters wherein scientific knowledge is required. In this informal capacity the Society has been requested to undertake the conduct of many enquiries in the public interest. It has been likewise entrusted with the administration of funds voted by Parliament for the promotion of investigation. To illustrate the character of this side of the Society's activity, let me cite some examples of the nature of the tasks which have been confided to it.

One of the most important of all these public functions is that of supervising the National Physical Laboratory. This great Institution, owing in large measure to the representations made by distinguished Fellows of the Society, was established by the State to secure accurate determinations and measurements of all kinds. Its ultimate control has been vested in the President and Council of the Royal Society, who nominate the Executive Committee which is entrusted with the immediate management of the

Laboratory and the determination of the nature of the work to be undertaken. The welfare and efficiency of this national establishment are thus closely associated with the Royal Society.

Another administrative control of public funds placed in our hands is that of the annual grant of £4000 voted by Parliament for scientific investigations. This money is sometimes ignorantly supposed to be a subsidy to the Royal Society itself, but our relation to it is entirely that of administration. Open to everyone who pursues science in this country, it has been instrumental in aiding and encouraging many workers who would otherwise have been unable to commence or to carry on their researches. In order to deal with the numerous claims made upon it, seven Boards have been constituted, each representing one of the great subdivisions of science. On these Boards upwards of fifty Fellows of the Society serve, selected for their eminence in their respective fields. Many of them are busy professional men; yet, entirely at their own charges, they come, in some cases from long distances in the country, to the meetings of the Boards here, and give much time to the consideration of the claims of the applicants. The average annual number of applications for grants during the last five years has been 107. Of these, the annual average accepted by the Boards during the same period is 85. Recipients of grants are bound to send in reports of their work and of the expenditure of the money voted to them. The average annual number of these reports which have been laid before the Boards during the last five years is 178. I doubt if the distribution of any other Parliamentary grant is more sedulously supervised than this appropriation for scientific investigations. But not only do the several Boards scrutinise the applications, they are empowered themselves to initiate researches on promising lines of enquiry, and have occasionally availed themselves of this prerogative.

Requests are not infrequently made to the Society by different Government Departments for advice or co-operation in matters wherein expert scientific knowledge is required. For years past we have had a Tropical Diseases Committee which, in association with the Colonial Office, has been carrying on investigations into the nature and prophylaxis of some of the maladies incident to the human and animal populations of our colonies and protectorates in warm climates. A Commission despatched by this Committee to Uganda has, for some time, been at work under Sir David Bruce studying the decimating scourge of sleeping sickness, while another Commission under the same Committee is busy in London searching experimentally for some drug that may be effective in the treatment of that terrible disease. A few years ago, at the joint instance of the War Office, Admiralty, and Colonial Office, we despatched a Commission to Malta to investigate the peculiar fever which had for so long a time reduced the effective strength of our garrisons and fleets in the Mediterranean. The observers were fortunate in soon discovering the source of the disease and were able to point out the steps to be taken to cope with it. The result has been that this serious malady has now been almost entirely banished from the hospitals of Malta. At present another Committee of the Society is engaged, at the request of the Home Office, in studying the disease known as glassworkers' cataract. The India Office likewise applies to us for advice, and we have an "Indian Government Advisory Committee" and an "Observatories Committee," the duty of which is to consider the reports of various public departments in the great dependency, and to offer suggestions towards the improvement of their scientific operations.

Another sphere of the Royal Society's activity lies in the administration of the various trusts which have been placed in our hands for scientific, educational, and charitable purposes. Of these trusts, one of the most important and useful is that known as the "Scientific Relief Fund," which was started in 1859 by Mr. J. P. Gassiot, and has been increased by the subsequent donations of many Fellows, notably Lord Armstrong, Sir William Siemens, and Mr. Bentham, until it now has an invested capital of about £18,000. The object of this fund is to aid, in an unobtrusive manner, such scientific men or their families as may from time to time require assistance. In the administration of its benefits regard is not confined to our own members, but grants are freely made to applicants from outside whose claims are supported by the President of some recognised scientific society.

One of the most honoured names in the history of the Royal Society is perpetuated in the "Donation Fund," established by William Hyde Wollaston, to which other benefactors have made additions. The interest of the investments thus provided furnishes the Council with the means of assisting researches and furthering the general purposes of the Society.

The "Gassiot Trust" has enabled us to aid in continuing the magnetical and meteorological observations which have given our observatory at Kew a world-wide reputation. This institution is now incorporated with the National Physical Laboratory, under conditions securing that the observational work for which it has long been so honourably known shall be maintained unimpaired.

By the "Mackinnon Research Fund" the Society has been enabled to establish studentships in natural and physical science, including geology, astronomy, and pathology, tenable by British subjects, after the completion of their University training. These appointments, which are renewable, may, in exceptional cases, be held for three years, and are of great usefulness in encouraging and aiding young men of promise who are starting upon a scientific career.

The "Joule Fund," which is international in character, is intended for the promotion of research by younger men in the branches of physical science in which Joule's discoveries were fundamental. The "Gunning Fund" was established for the furtherance of knowledge in physics and biology.

During the present year three additional funds have been constituted in accordance with the wishes of three deceased Fellows. By the foundation of the "Sorby Fund" the Royal Society is entrusted with the administration of the interest of a sum of £15,000 for the purpose of creating and maintaining a professorship or fellowship for scientific research, to be associated primarily with the University of Sheffield. The first appointment to this post was recently made by a Committee representing the Society and the University. Another well-remembered name will now be perpetuated among the activities of the Royal Society by the "Tyndall Mining Research Fund," consisting of £1000 which has been given by Mrs. Tyndall, in accordance with her husband's desire, for furthering research in all matters pertaining to mining and the welfare of miners. The "Gore Bequest," already referred to, has placed in our hands a sum of upwards of £2400, the interest of which is to be devoted to assist original scientific discovery.

Although the Royal Society administers annually a considerable sum of money, it will be observed that by far the largest part of the disbursements is ear-marked for various special applications, and cannot be employed for other objects. So far, indeed, as its general purposes are concerned, the Society cannot be regarded as adequately provided. For nearly two hundred and fifty years it has continued to hold aloft the torch of science, but the constantly augmenting demands of modern progress make its task increasingly difficult of satisfactory performance. I have referred to the growing cost of our publications, and there are other parts of our organisation wherein the development of our work is hampered by the lack of funds. Men of science are seldom rich; it is therefore all the more gratifying to be able to record examples of the continuous generous liberality of our Fellows. But it is hardly from our own ranks that we can look for any substantial addition to our resources. Perchance in the general community there may yet be found some men who may be led to see that besides the various laudable objects that have hitherto claimed their care, the advancement of science is likewise an important public and educational interest, and that benefactions are not unworthily bestowed in enabling the Royal Society adequately to maintain the great work which it has inherited from the past.

MEDALLISTS, 1909.

COPLEY MEDAL.

The Copley Medal is this year awarded to Dr. George William Hill, For.Memb.R.S. Now that Simon Newcomb is no longer with us, Dr. Hill occupies, beyond challenge, the first position in the great subject of Dynamical Astronomy.

His processes are not only marked by extraordinary originality, the result of high mathematical genius, but also in every case his methods and researches are directed towards practical astronomical ends. His supreme work is probably contained in his researches on the theory of the moon's motion, which has remained the great problem of gravitational astronomy ever since the time of Newton. Here his introduction and development of the principle of disturbed periodic orbits has given an entirely new direction to the science, culminating recently in the Lunar Tables of E. W. Brown, which mark an epoch in the practical side of the Lunar Theory.

This work of Hill has been fruitful in new advances in many directions. His ideas have given rise, as developed by Poincaré and other investigators, to new departments of abstract mathematical analysis, while in the hands of Lord Rayleigh they have shed light on important and difficult problems of general mathematical physics.

His collected works have recently been published by the Carnegie Institution of Washington in four quarto volumes; the importance of their contents can hardly be over estimated. M. Henri Poincaré, in his introduction to these volumes, described Hill as "une des physionomies les plus originales du monde scientifique américain."

Astronomy owes to him new theories of the motions of the systems of Jupiter and Saturn, to which the whole of Vol. III of his works is consecrated.

His shorter papers deal with nearly every problem in the Lunar and Planetary theories, with mathematical geodesy and other subjects. All his work is characterised by its original points of view combined with practical aims, by maturity of thought and high suggestiveness. It forms an index of the simplicity and aloofness of its author, who has been one of the main ornaments of Astronomical Science for more than a generation.

ROYAL MEDALS.

One of the Royal Medals has been awarded, with the approval of His Majesty the King, to Prof. Augustus Edward Hough Love, F.R.S., in recognition of his numerous and important contributions to mathematics, and especially to mathematical physics. He has written many valuable papers on various branches of hydrodynamics, in particular on the theories of jets, of vortex motion, and of revolving gravitating masses of liquid. He is the author of a work on "Elasticity," now in its second edition, which is highly appreciated at home and abroad, and ranks as the standard treatise on the subject. In this he has incorporated various valuable researches of his own, which have appeared in the 'Philosophical Transactions' and elsewhere. He has further investigated closely the circumstances of wave-propagation in air, in elastic solids, and in the electromagnetic medium, and has examined in particular the phenomena which present themselves at wave-fronts when the motion is discontinuous. More recently he has published remarkable papers on terrestrial physics, including a speculation on the origin of the present distribution of land and water, and an investigation of the precise extent of the inferences which can be drawn as to the internal constitution of the earth from the observed *data* relating to the heights of ocean tides of long periods, the lunar disturbance of level, and the approximate period of the small movements of the Pole over the earth's surface.

His Majesty has likewise approved of the award of the other Royal Medal to Major Ronald Ross, F.R.S.

The name of Major Ross has become widely known on account of the important investigations which he has carried out on the life-history of the malarial organism and the means of preventing malarial infection. Following up a clue indicated by Manson, he began, in 1895, at Secunderabad, in India, under circumstances which entailed much difficulty and many delays, an investigation as to whether the malaria parasite, discovered by Laveran, passes part of its life-history within the body of a biting insect. After more than two years of fruitless experiments Ross discovered a stage of the human malaria parasite in the tissues of a mosquito (*Anopheles*) which had been allowed to feed on the blood of a malarial patient. In 1898 he proceeded to work out in detail the life-history of a malarial parasite found in sparrows and larks in India. He traced the complicated stages in the development of this parasite from its inception into the stomach of a gnat (*Culex fatigans*), which feeds on the blood of these birds, to its passage back into their blood through the secretion of the poison gland of the insect. At the same time he furnished conclusive experimental proof of the part played by the insect in propagating the infection. These fundamental observations have been confirmed and extended in various directions by other observers, both in the British Empire and elsewhere.

As a practical consequence of the discoveries of Ross and those who have followed in his footsteps, and of his own unceasing exertions and further investigations during the last few years, scientifically directed measures for the prevention of malaria have been initiated with striking success in many fever-stricken districts all over the world, and particularly within the British Empire. His investigations have also inspired similar work on the spread, by means of mosquitoes or other biting insects, of other formidable diseases, with the result that effective measures have been devised for preventing the spread of these diseases also.

DAVY MEDAL.

The Davy Medal has been awarded to Sir James Dewar, F.R.S.

Sir James Dewar has been a pioneer in the study of very low temperatures, their production, applications, and effects.

For many years he has worked continuously in this difficult domain, and his investigations have resulted from time to time in such achievements as the solidification of oxygen, the liquefaction of fluorine, and the liquefaction and solidification of hydrogen. His improvements in technique have been fundamental. By the construction of vessels in which thermal convection is avoided by the presence of a vacuous layer in their walls, he has enormously simplified the retention and manipulation of matter at very low temperatures. His application of the absorbent effect exerted on gaseous materials by charcoal at low temperatures has placed in the hands of chemists and physicists a most convenient and important method, not only for the production of high vacua, but also for the rapid separation of the constituents of gaseous mixtures. The modifications in the properties of matter at very low temperatures have been investigated, and remarkable results obtained, including the earliest exact investigations, jointly with Prof. Fleming, on the electric properties of insulators, and of metals and alloys. The determination of the properties (critical points, boiling points, etc.) of refractory gases at very low temperatures has involved the practical downward extension of absolute thermometry, with the result that temperatures in the neighbourhood of the absolute zero can be determined correctly to within a degree. Lastly, recent measurements of the rate of formation of helium from radium salt, specially purified by Sir T. Edward Thorpe for his recent atomic weight determination, have provided exact molecular data, throwing light on the nature of the spontaneous disintegration of that very remarkable substance.

HUGHES MEDAL.

The Hughes Medal falls this year to Richard Tetley Glazebrook, F.R.S.

Dr. Glazebrook has for many years been closely identified with the construction, testing, and evaluation of electrical standards. Not only has he published important memoirs on these subjects, but, as Secretary for a very long period of the Electrical Standards Committee of the British Association, and more recently as Director of the National Physical Laboratory, he has taken a leading and responsible part in this type of scientific work and in conferences of international importance. It is thus specially fitting that he should be the recipient of the Hughes Medal.

The Velocity of Reaction in the "Absorption" of Specific Agglutinins by Bacteria, and in the "Adsorption" of Agglutinins, Trypsin, and Sulphuric Acid by Animal Charcoal.[*]

By GEORGES DREYER, M.A., M.D., Professor of Pathology in the University of Oxford, and J. SHOLTO C. DOUGLAS, M.A., B.M., Philip Walker Student in Pathology in the University of Oxford, formerly Radcliffe Travelling Fellow.

(Communicated by Prof. F. Gotch, F.R.S. Received November 16, 1909,—Read January 20, 1910.)

(From the Department of Pathology, University of Oxford.)

Arrhenius, in his different papers dealing with equilibria in absorption processes, has used the alleged differences in velocity of reaction between the absorption of agglutinin by bacteria and the adsorption of a dye by a fibre, to assist his conclusion that these processes are of different natures.

While a fair number of observations (Bordet, Bayliss, Hedin, etc.) exist as to the influence of time on the so-called adsorption processes, *e.g.*, the adsorption of an acid by charcoal, or of a dye by a fibre, proving that it takes a very considerable time before equilibrium is reached, the study of the time reaction in the taking up of agglutinins by bacteria has been confined to the following brief statements of Eisenberg and Volk, that the velocity of reaction is extremely fast and that equilibrium is reached even in five minutes at a temperature of 0° C., and that no appreciable difference was to be traced in the absorption velocity whether the reaction took place at 0° C. or at 37° C.

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[*] The experiments in this paper were partly carried out in the University Laboratory for Medical Bacteriology, Copenhagen, and we wish to express our gratitude to Prof. Salomonsen, the Director of that Laboratory, for the great facilities he always granted us while we worked there.

Arrhenius, having founded his theory for the equilibria in absorption processes on the experiments of Eisenberg and Volk, uses the statement that, as equilibrium is reached in less than five minutes even at 0° C., the process of the taking up of agglutinin by bacteria could not be an adsorption similar to the interaction between dye and fibre as proposed by Bordet, since the reaction velocity is here very slow, taking a long time, even days, before equilibrium is reached at room temperature.

We made the following series of experiments to determine for ourselves if the statements of Eisenberg and Volk were correct, especially as they were so much in opposition to what one would *a priori* expect. We wished to find out whether it would be possible, by comparing the velocity of absorption of specific agglutinins by their own bacteria with the velocity of adsorption of the same specific agglutinins and other organic and inorganic substances as, *e.g.*, trypsin or sulphuric acid by charcoal, to draw definite conclusions as to the difference in the two processes in the manner done by Arrhenius to support his theory that the interaction between specific agglutinin and bacteria is only a special case of Guldberg and Waage's law of mass action.

Before passing on to our own results, we must give a detailed account of the technique which enabled us to reach more trustworthy results than could be expected by the somewhat rough procedure of Eisenberg and Volk.

Technique.—For the experiments we used a brand of *Bacillus coli communis* (called in the tables *Bacillus coli lab.*) the purity of which could be relied upon and which had been long subcultured in the laboratory, being the same as that previously used by Dreyer and Jex-Blake. The emulsions were made from cultures grown for 24 hours in large Roux bottles on agar surfaces inoculated from a 24-hour bouillon culture which had been transplanted daily from bouillon to bouillon for about a week. The surface growth was removed with an aluminium scraper, great care being exercised to include neither the agar itself nor the water of condensation. The mass so obtained was emulsified by rubbing it with a glass rod in a glass beaker, 0·85 per cent. NaCl being gradually added, until the emulsion was of the required density. This was such that the emulsion contained 100 times as many bacilli as the standard bouillon culture used for testing the agglutinin content of any given serum, and was called 100/1 N emulsion. The standardisation was effected by comparing its translucency with that of the standard culture, a method which allows of great accuracy.

To the 100/1 N emulsion was added 1 per cent. of ordinary commercial 40 per cent. formaldehyde. This stock emulsion was kept in an ice chest in the dark at from 0° to 2° C. Usually a 10/1 N emulsion was used for the absorption of the agglutinin, the stock emulsion being suitably diluted with sterile 0·85 per cent. NaCl for each experiment. Control experiments were made to ascertain that formalin in this amount did not give appreciable differences in the quantity of agglutinin absorbed. The experimental details on this point will be published in a later paper.

The serum used, unless otherwise stated, was obtained from a goat immunised with the stock *Bacillus coli* mentioned above, and was suitably diluted with sterile 0·85 per cent. NaCl. All apparatus used was carefully cleaned and dry sterilised. All measurements and dilutions were carried out with accurately graduated glass pipettes. For investigating the absorption of agglutinin by bacteria, 4 c.c. of the emulsion of the corresponding bacilli were added to 4 c.c. of a given dilution of serum. The tubes were then at once shaken and left to stand for various lengths of time.

Experiments were undertaken at low temperature (1° to 2° C.), at room temperature (15° to 17° C.), and in one case at 37° C., room temperature being that to which preference was given, because here no, or only slight, alteration in the temperature took place during the time of centrifugalisation, while with the experiments at 1° to 2° C. it was found impracticable to prevent the temperature from rising during this period, as also to prevent a fall in temperature if the experiments were carried out at 37° C.

A special high speed centrifuge by Burmeister and Wain, run at a speed of 7200 revolutions per minute for 10 minutes, was used for the separation of the bacteria from the fluid. The clear supernatant fluid was decanted and kept in the dark at a temperature of 0° to 2° C. until it was convenient, on the same or the following day, to estimate the amount of agglutinin it contained.

The measurements of the agglutinin content of the various fluids were carried out by Dreyer's modification* of the method originally proposed by Madsen and Jörgensen.†

The method adopted for the estimation of agglutinin is as follows:— Test-tubes 7 cm. long and 1 cm. in diameter were arranged in sets or series of 13, and into each tube were measured out the following quantities of the various fluids employed (Table I):—

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* Dreyer and Jex-Blake, 'Trans. Royal Danish Academy,' 1905.

† 'Festskrift ved Indvielse af Statens Seruminstitut, Copenhagen,' 1902.

Table I.

Tube.	0·85-per-cent. NaCl, in c.c.	Diluted agglutinating serum, in c.c.	Standardised killed bouillon culture, in c.c.
1	0·0	1·0	1·5
2	0·2	0·8	1·5
3	0·4	0·6	1·5
4	0·5	0·5	1·5
5	0·6	0·4	1·5
6	0·65	0·35	1·5
7	0·7	0·3	1·5
8	0·75	0·25	1·5
9	0·8	0·2	1·5
10	0·83	0·17	1·5
11	0·87	0·13	1·5
12	0·9	0·1	1·5
13	1·0	0·0	1·5

Each series of tubes thus contains quantities of agglutinating serum or other fluid to be tested, diminishing from 1 c.c. to 0·1 c.c., Tube 13 acting as a control to ensure the absence of spontaneous agglutination.

As soon as all these measurements were completed the tubes were shaken from right to left, care being exercised not to carry fluid from one tube to the other. They were then placed in a water bath at 37° C. for two hours, taken out, allowed to cool down to room temperature (which takes about a quarter of an hour), and examined for agglutination. Thereafter they stood a further 20 to 24 hours at room temperature and were again examined.

When the agglutinating powers of several sera are to be examined, a similar series of 13 test-tubes is set up for each. The actual measurement of the agglutination that has taken place is made as follows:—From the whole number of tubes set up as above described, that one is selected which shows the minimum amount of agglutination definitely visible to the naked eye. This tube is called the "standard" tube, and it is carefully compared with those tubes in each of the other series which show nearly similar degrees of agglutination. In this way one is able either to pick out in each series a tube showing the same degree of agglutination as the standard, or to estimate by visual inspection the situation that such a tube should occupy between one tube showing a greater degree of agglutination and the next tube in the series showing a less degree of agglutination than the standard tube.

Taking the reciprocal figure of the actual amount of diluted serum added to the tube giving the same degree of agglutination as the standard tube, and multiplying this figure by the number of times the serum was diluted, it is possible to express the agglutinin strength of the serum in arbitrary units. Thus it will be seen that it is possible to measure the agglutinin content of a given serum, whether it is strong or weak, with the same procentic accuracy, *i.e.* to determine whether a serum contains 1 or 1·1 unit as accurately as if it contained 1000 or 1100 units.

The digesting power of the trypsin used in one set of experiments was measured in a similar manner. A slightly alkaline 10 per cent. gelatin jelly, to which had been added a trace of thymol, took the place of the bacterial culture, the trypsin solution that of the diluted serum. After the fluid contents had been measured into the tubes, these were shaken and placed in a water bath at 37° C. for two hours and then placed in a cold water bath (8° to 10° C.) to solidify, and read while still in the bath. A second reading was taken 24 hours later to make sure that the end reaction had actually been reached. A tube which showed semi-liquefaction was chosen as standard in reading the result.

As we, in the course of our investigations, have been convinced of the importance of paying attention to points previously raised by Dreyer and Jex-Blake, it may be of use to summarise the principal differences between the method as used by us throughout our experiments, and the method as originally proposed by Madsen and Jörgensen.

1. To use a standardised killed bouillon culture as described by Dreyer* instead of a fresh 24-hour bouillon culture, of non-standardised strength, to which 0·02 per cent. of formalin had been added immediately before use in order to prevent the further growth of the bacteria while the tubes were kept for two hours at 37° C.

2. To make the contents of each tube up to the same volume. Madsen and Jörgensen, and also Jörgensen, have stated that variations in the same amounts of fluid in their test-tubes lying between 1·56 c.c. and 2 c.c. made no difference in the results they obtained, any alterations due to such variations lying within the limits of experimental error. Our own experience, however, is entirely opposed to this view.

3. To take two separate readings of the tubes: the first after they have been left two hours at 37° C. and allowed to cool to room temperature, and the second after they have been left a further 20 to 24 hours at room temperature. This is preferable to the reading of the tubes as soon as they are removed from the water bath and before they have had time to cool down to the temperature of the room as proposed by Madsen and Jörgensen, a course which is impossible where many series of tubes have to be examined, and also, in our experience, gives less trustworthy results. We find that the

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* 'Hospitalstidende,' No. 19, 1906, and 'Journ. of Path. and Bact.,' vol. 1, 1909.

24-hour reading is in nearly all cases the one to which the greatest attention should be paid, because such irregularities as are found in the two-hour reading in the same serum in the same dilution (being caused by some slight difference in the physical condition under which the clumping of the bacteria takes place, time reaction, etc.) will generally totally vanish in the 24-hour reading. This absence of irregularities is of the utmost importance where great accuracy is essential, as, for instance, in absorption experiments. The following figures (Table II) clearly illustrate the advantage of the 20- to 24-hour reading.

Table II.—Figures (reduced to terms of a 100 in all cases for ease of comparison) showing the advantage of examining the tubes 20 to 24 hours after, instead of immediately after they had been taken from the water bath. The series recorded in each different experiment were made up in an exactly similar manner.

Experiment.	Date.	Series.	After 2 hours at 37° C.		After a further 20 to 24 hours at room temperature.
			Coarse reading.	Fine reading.	
A	30.4.07	1	100	100	100
		2	130	112	100
B	4.5.07	1	100	100	100
		2	121	132	97
C	7.5.07	1	100	100	100
		2	116	102	100
D	18.5.07	1	100	100	100
		2	111	109	103·5
		3	111	109	103·5
E	2.6.07	1	100	100	100
		2	108	100	100
		3	108	104	100·6
F	12.6.07	1	100	100	100
		2	108	108	101
		3	122	112	101
G	23.8.07	1	100	100	100
		2	100	106	103·5
		3	112	111	100
H	1.10.07	1	100	100	100
		2	120	131	102
		3	137	129	102
		4	122	120	100
K	21.10.07	1	100	100	100
		2	128	113	99
		3	112	106	101

Turning next to the "Tables" at the end of this paper, it should be explained that when it is stated in any one of them that a given agglutinating serum, or a solution of trypsin or of sulphuric acid, contains a certain number of "units," these "units" may be taken as expressing absolute values, though the "units" themselves are arbitrary. This is because control measurements were carried out at the time in each set of experiments, and the number of "units" present both before and after absorption has taken place for a given time have been determined by the use of the same arbitrary standard.

With experiments carried out with identical solutions or emulsions, but made at different times, the results are obtained in values which are relative and not absolute, and so cannot be directly compared with one another. Accordingly in Tables III to XIV the experimental details and the absolute quantities employed are recorded. In Table XV all the results are summarised, but have been recalculated so as to give figures that are directly comparable with one another.

These figures express relative and not absolute values; in each case the number of "units" originally present has been taken as 100, while the quantities absorbed and the quantities left free in the fluid are expressed in corresponding ratios or percentages.

To the time of observation given in the tables must be added the interval of time elapsing between the mixing of the bacteria with the serum and the stopping of the centrifuge, less an unknown time X. This subtraction of the time X is necessary, because it is impossible to say at what precise moment during the centrifugalisation the absorbing material was completely separated from the supernatant fluid, and it is this moment which we regard as the end of the reaction. Thus the time must be read as

$$0+(\text{time}-X),\ 1+(\text{time}-X),\ \ldots\ 20+(\text{time}-X), \text{ etc., minutes.}$$

Naturally the length of time X may be different according to the nature, quantity, etc., of the absorbing matter used, *i.e.* bacteria, charcoal, etc., and the concentration, nature, etc., of the fluid with which it is in contact, but is constant throughout each individual experiment since these conditions then remain the same.

In the following discussion, when mentioning the various times, we speak of 0 minutes instead of $0+(\text{time}-X)$ minutes, 3 minutes instead of $3+(\text{time}-X)$ minutes, etc., for the sake of brevity.

In Experiment 1 (Table III), where there were originally present 197·5 units, the number of units left in the fluid decreases regularly as the time increases, although the experiment was carried out at a temperature higher than 0° C., *i.e. circa* 1° C. Thus it will be seen that after 0 minutes 19 units are left, after 10 minutes 7·9, and after 80 minutes 5·4 units.

Somewhat similar results are found in Experiment 2 (Table IV) carried out at 2° C., where, out of 162 units, there were left after 0 minutes 12·5 units, after 4 minutes 9·7, and after 20 minutes 6·06.

It is thus clearly seen that at temperatures but little higher than 0° C., equilibrium is by no means established even in times of more than 30 minutes. This is in no sort of agreement with the statement of Eisenberg and Volk which has been generally accepted by Arrhenius, Craw, and others.

As it now was of interest to see whether equilibrium would be established in a short time at higher temperatures, such as 15° to 17° C. and 37° C., Experiments 3 and 4 (Tables V and VI) were carried out. In Experiment 3, carried out at 37° C., the serum diluted 12·5 times contained 29·2 units. After 0 minutes were left 3·49 units, after 9 minutes 2·27, after 30 minutes 2 units, but that equilibrium had not been reached even after half an hour is seen from the fact that after 2, 4, and 8 hours there has been left respectively 1·85, 1·78, and 1·59 units. Exactly the same is found in Experiment 4, carried out at 16° C., as will be seen from Table VI, save that the velocity of reaction is slower than in Experiment 3.

To show with how great accuracy experiments of this kind can be carried out, and to prove beyond doubt the correctness of the statements, in each of the Experiments 3 and 4 there were put up three tubes for the time denoted as 0 minutes, and two for the time denoted as 9 minutes. It is clear that in Experiment 3, where the greatest individual differences between the observed values are found, the mean variation is, nevertheless, not greater than 3·1 per cent. from the mean value; and for 9 minutes the two determinations are identical. In Experiment 4, for the 0 minute point the mean variation is less than 0·9 per cent. of the mean value, and at 9 minutes, just as in the previous experiment, the figures are identical. As one would expect, the greatest error is found where the time reaction is the shortest, and is due to the inevitable small differences in time which can hardly be avoided. Similar control tubes were used in Experiments 7, 8, 9 (Tables IX, X, XI).

These experiments clearly show that one of the fundamental points stated by Arrhenius to be in good agreement with his theory that the absorption of agglutinin by bacteria should be considered as following a partition law, $C = kB^n$, similar to that which holds good for the distribution of benzoic acid between two different solvents, water and benzene, is unsound, since the reaction, instead of being a very rapid one (finished in less than five minutes at 0° C., Eisenberg and Volk), is a slow one and is still incomplete at room temperature even after several hours have elapsed.

As we know from experiments of Morgenroth that it takes several hours at room temperature before equilibrium is reached, when a diphtheria antitoxin acts on a diphtheria toxin, there is now no excuse for contrasting "the rapid reaction" in the one case (absorption of agglutinin) with "the slow reaction" in the other (neutralisation of diphtheria toxin). To draw such a distinction between the interactions of these different antigens and antibodies is unjustifiable, since in both instances the reaction is rather a slow one. As Arrhenius has used "the rapid reaction" in the case of the absorption of agglutinins by bacteria to disprove Bordet's theory that the agglutinins were adsorbed by the bacteria in the same way as a dye by a fibre, where the reaction is a slow one, it was of interest to make experiments which would enable one to compare the velocity of reaction in adsorption processes with that found by us in the absorption of agglutinin by bacteria. For this purpose we have investigated the adsorption of agglutinins, trypsin, and sulphuric acid by animal charcoal.

From Experiments 5, 6, 7, 8 (Tables VII, VIII, IX, X), in which agglutinin is acted on by charcoal, it will be seen that the amount of agglutinin left free in the supernatant fluid lessens as the time during which the adsorption is allowed to proceed increases. This observation is in entire agreement with those made by previous observers, showing that in adsorption processes it takes a long time before equilibrium is reached.

Comparing together Experiments 5 and 6, it is further seen that, at the same temperature, where small amounts of charcoal acted on small amounts of agglutinin, the velocity of reaction is smaller than with larger amounts of charcoal and larger amounts of agglutinin, within the limits of the experiments.

From Experiments 5, 6, 7, and 8, it results that the rate with which the adsorption of agglutinin by charcoal proceeds is markedly greater than is that of the absorption of agglutinin by bacteria. But if we compare the progress of adsorption in these experiments with that seen in Experiment 9 (Table XI), in which trypsin is treated with charcoal, it is clear that the rate of adsorption in this latter case is ever so much the greater.

Examination of Experiments 10, 11, and 12 (Tables XII, XIII, XIV), in which charcoal is allowed to adsorb sulphuric acid, shows that the quantity of acid adsorbed augments with the lapse of time, but does so less rapidly than was the case in the other adsorption experiments (Nos. 5 to 9). The rate of adsorption is, in fact, approximately the same as it was in the absorption experiments (Nos. 1 to 4), in which agglutinins were placed in contact with bacilli. For example, in Experiment 4 (Table XV), the percentage of agglutinin absorbed increased from 82·2 at the end of

0 minutes to 92·0 after 240 minutes; in the case of charcoal and sulphuric acid (Experiment 12, Table XV), the percentage adsorbed rose from 58·7 at the end of 0 minutes to 65·4 at the end of 240 minutes.

Thus it is found that the time taken before equilibrium is reached varies greatly, not only with the quantity of the adsorbing matter but also with the nature of the different substances, concentrations, absolute quantities, temperature, etc., and that it is not legitimate to draw from the time taken for equilibrium to be reached any definite conclusion as to the nature of the interaction between the absorbing substance and the substance absorbed from a fluid.

Therefore we consider it just as erroneous to conclude from the reaction velocities that the taking up of agglutinin by bacteria is the same process as the taking up of sulphuric acid by charcoal (Bordet's theory) as it would be to conclude that the adsorption of trypsin by charcoal or agglutinin by charcoal is different from the adsorption of sulphuric acid by charcoal, on account of the great differences in the velocities with which these processes proceed.

Table III.*—Experiment 1. May 3, 1907.

Temperature, 1° C. Serum, Kringelgaard horse serum, 16 per cent. Bacilli, 10/1 N *Bacillus coli communis* (Kringelgaard), April 24, 1907. Time taken in centrifuging, etc., 32 minutes.

Time in minutes.	B.	C.
$(32-x)+0$	19	178·5
„ 10	7·9	189·6
„ 20	6·6	190·9
„ 30	5·9	191·6
„ 40	5·7	191·8
„ 80	5·4	192·1
T = 197·5		

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* In every case, the terms T, B, and C will be used in the following sense:—

T = the total number of units which the given dilution of serum contained before absorption.

B = the number of units left free in the supernatant fluid after absorption has taken place.

C = T minus B, *i.e.* the number of units removed from the original solution by the absorbing matter.

Table IV.—Experiment 2. May 7, 1907.

Temperature, 2° C. Serum, Kringelgaard horse serum, 16 per cent. Bacilli, 10/1 N *Bacillus coli communis* (Kringelgaard), April 24, 1907. Tme in centrifuging, etc., 28 minutes.

Time in minutes.	B.	C.
$(28-x)+0$	12·5	149·5
,, 4	9·7	152·3
,, 8	7·7	154·3
,, 12	6·7	155·3
,, 20	6·06	155·94
	T = 162	

Table V.—Experiment 3. October 29, 1907.

Temperature, 37° C. Serum, *Coli lab.* (goat), June 16, 1907, 8 per cent. Bacilli, 10/1 N *Coli lab.* emulsion, June 26, 1907. Time taken in centrifuging, etc., 25 minutes.

Time in minutes.	B.	C.
$(25-x)+0$	$3{\cdot}49\left\{\begin{array}{l}3{\cdot}57\\3{\cdot}57\\3{\cdot}33\end{array}\right.$	25·71
,, 1	3·25	25·95
,, 3	2·82	26·38
,, 5	2·59	26·61
,, 9	$2{\cdot}27\left\{\begin{array}{l}2{\cdot}27\\2{\cdot}27\end{array}\right.$	26·93
,, 11	2·18	27·02
,, 13	2·16	27·04
,, 15	2·13	27·07
,, 30	2·00	27·20
,, 60	1·96	27·24
,, 120	1·85	27·35
,, 240	1·78	27·42
,, 480	1·59	27·61
	T = 29·20	

Table VI.—Experiment 4. October 16, 1907.

Temperature, 16° C. Serum, *Coli lab.* (goat), June 16, 1907, 8 per cent. Bacilli, 10/1 N *Coli lab.* emulsion, June 26, 1907. Time taken in centrifuging, etc., 28 minutes.

Time in minutes.	B.	C.
$(28-x)+$ 0	5·07 $\left\{\begin{matrix}5{\cdot}00\\5{\cdot}10\\5{\cdot}10\end{matrix}\right.$	23·34
,, 1	4·57	23·84
,, 3	4·24	24·17
,, 5	4·00	24·41
,, 7	3·86	24·55
,, 9	3·70 $\left\{\begin{matrix}3{\cdot}70\\3{\cdot}70\end{matrix}\right.$	24·71
,, 11	3·62	24·79
,, 13	3·56	24·85
,, 15	3·46	24·95
,, 20	3·36	25·05
,, 30	3·11	25·30
,, 60	2·72	25·69
,, 120	2·42	25·99
,, 240	2·27	26·14
,, 420	2·13	26·28
T = 28·41		

Table VII.—Experiment 5. September 14, 1907.

Temperature, 16°·5 C. Serum, *Coli lab.* (goat), May 6, 1907, 0·5 per cent. Animal charcoal, 0·04 gramme. Time taken in centrifuging, etc., 28 minutes.

Time in minutes.	B.	C.
$(28-x)+$ 0	3·3	2·34
,, 5	2·88	2·76
,, 10	2·67	2·97
,, 15	2·56	3·08
,, 20	2·5	3·14
,, 30	2·44	3·20
T = 5·64		

Table VIII.—Experiment 6. September 20, 1907.

Temperature, 16° C. Serum, *Coli lab.* (goat), May 6, 1907, 4 per cent. Animal charcoal, 0·5 gramme. Time taken in centrifuging, etc., 29 minutes.

Time in minutes.	B.	C.
$(29-x)+0$	9·8	30·35
„ 5	6·9	33·15
„ 10	5·99	34·16
„ 15	5·4	34·75
„ 20	5·26	34·89
„ 30	4·8	35·35
	T = 40·15	

Table IX.—Experiment 7. October 1, 1907.

Temperature, 16° C. Serum, *Coli lab.* (goat), June 16, 1907, 4 per cent. Animal charcoal, 0·5 gramme. Time taken in centrifuging, etc., 30 minutes.

Time in minutes.	B.	C.
$(30-x)+0$	$8{\cdot}97\begin{cases}9{\cdot}1\\8{\cdot}9\\8{\cdot}9\end{cases}$	20·53
„ 1	8·7	20·8
„ 3	8·3	21·2
„ 5	8·1	21·4
„ 7	7·9	21·6
„ 9	$7{\cdot}6\begin{cases}7{\cdot}5\\7{\cdot}7\end{cases}$	21·9
„ 11	7·1	22·4
„ 13	6·9	22·6
„ 15	6·7	22·8
„ 20	6·0	23·5
„ 30	4·7	24·8
„ 60	3·7	25·8
„ 120	3·06	26·44
„ 240	2·50	27·0
„ 480	1·77	27·73
	T = 29·5	

Table X.—Experiment 8. October 7, 1907.

Temperature, 16° C. Serum, *Coli lab.* (goat), June 16, 1907, 4 per cent. Animal charcoal, 0·5 gramme. Time taken in centrifuging, 28 minutes.

Time in minutes.	B.	C.
$(28-x)+0$	$8{\cdot}63\left\{\begin{array}{l}8{\cdot}6\\8{\cdot}7\\8{\cdot}6\end{array}\right.$	18·77
„ 1	8·0	19·4
„ 3	7·5	19·9
„ 6	6·8	20·6
„ 9	5·9	21·5
„ 12	$4{\cdot}95\left\{\begin{array}{l}5{\cdot}0\\4{\cdot}9\end{array}\right.$	22·45
„ 16	4·4	23·0
„ 20	4·2	23·2
„ 25	4·05	23·35
„ 30	$3{\cdot}95\left\{\begin{array}{l}3{\cdot}9\\4{\cdot}0\end{array}\right.$	23·45
„ 60	3·35	24·05
„ 120	2·90	24·50
„ 240	2·17	25·23
„ 480	1·35	26·05

T = 27·4

Table XI.—Experiment 9. October 26, 1907.

Temperature, 13°·5 C. Trypsin (Grübler), 1·25 per cent. solution. Animal charcoal, 0·1 gramme. Time taken in centrifuging, etc., 30 minutes.

Time in minutes.	B.	C.
$(30-x)+0$	$152\left\{\begin{array}{l}145\\167\\143\end{array}\right.$	1020·5
„ 1	138	1034·5
„ 3	115	1057·5
„ 5	106	1066·5
„ 12	50	1122·5
„ 15	37	1135·5
„ 20	23	1149·5
„ 30	14	1158·5
„ 60	7·3	1165·2
„ 120	3·26	1169·24
„ 240	1·82	1170·68
„ 420	1·15	1171·35

T = 1172·5

Table XII.—Experiment 10. July 2, 1908.

Temperature of room. Sulphuric acid, 1/40 N solution. Animal charcoal, 0·8 gramme. Time taken in centrifuging, etc., 30 minutes.

Time in minutes.	B.	C.
$(30-x)+$ 0	1·66	2·375
" 12	1·57	2·465
" 24	1·517	2·518
" 36	1·48	2·555
" 48	1·46	2·575
" 60	1·44	2·595
T = 4·035		

Table XIII.—Experiment 11. July 3, 1908.

Temperature of room. Sulphuric acid, 1/40 N solution. Animal charcoal, 0·8 gramme. Time taken in centrifuging, etc., 31·5 minutes.

Time in minutes.	B.	C.
$(31·5-x)+$ 0	1·606	2·368
" 12	1·499	2·475
" 24	1·446	2·528
" 36	1·416	2·558
" 48	1·391	2·583
" 60	1·379	2·595
T = 3·974		

Table XIV.—Experiment 12. July 9, 1908.

Temperature of room. Sulphuric acid, 1/40 N solution. Animal charcoal, 0·8 gramme. Time taken in centrifuging, etc., 28 minutes.

Time in hours.	B.	C.
$[(28-x)$ mins.$]+$ 0	1·67	2·375
" 0·6	1·478	2·567
" 2	1·424	2·621
" 4	1·399	2·646
" 12·5	1·344	2·701
" 24	1·286	2·759
" 48	1·141	2·904
T = 4·045		

Table XV.

	Agglutinin and bacilli.								Agglutinin and charcoal.								Trypsin and charcoal.		Sulphuric acid and charcoal.					
	Exp. 1. 3.5.07.		Exp. 2. 7.5.07.		Exp. 3. 29.10.07.		Exp. 4. 16.10.07.		Exp. 5. 14.9.07.		Exp. 6. 20.9.07.		Exp. 7. 1.10.07.		Exp. 8. 7.10.07.		Exp. 9. 26.10.07.		Exp. 10. 2.7.08.		Exp. 11. 3.7.08.		Exp. 12. 9.7.08.	
Temp. =	1° C.		2° C.		37° C.		16° C.		16°·5 C.		16° C.		16° C.		16° C.		13°·5 C.		"Room."		"Room."		"Room."	
Time +	(32−*x*) mins.		(28−*x*) mins.		(25−*x*) mins.		(28−*x*) mins.		(28−*x*) mins.		(29−*x*) mins.		(30−*x*) mins.		(28−*x*) mins.		(30−*x*) mins.		(30−*x*) mins.		(31·5−*x*) mins.		(28−*x*) mins.	
	B.	C.	B.	C.	B.	C.	B.	C.	B.	C.	B.	C.	B.	C.	B.	C.	B.	C.	B.	C.	B.	C.	B.	C.
0 mins.	9·63	90·37	7·72	92·28	11·97	88·03	17·8	82·2	58·4	41·6	24·4	75·6	30·4	69·6	31·5	68·5	13·0	87·0	41·2	58·8	40·1	59·6	41·3	58·7
1 ,,	—	—	—	—	11·15	88·85	16·1	83·9	—	—	—	—	29·5	70·5	29·2	70·8	11·8	88·2	—	—	—	—	—	—
3 ,,	—	—	—	—	9·66	90·34	15·9	84·1	—	—	—	—	28·2	71·8	27·4	72·6	9·83	90·17	—	—	—	—	—	—
4 ,,	—	—	5·99	94·11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
5 ,,	—	—	—	—	8·89	91·11	14·1	85·9	51·0	49·0	17·2	82·8	27·4	72·6	—	—	9·06	90·94	—	—	—	—	—	—
6 ,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	24·8	75·2	—	—	—	—	—	—	—	—
7 ,,	—	—	—	—	—	—	13·57	86·43	—	—	—	—	26·8	73·2	—	—	—	—	—	—	—	—	—	—
8 ,,	—	—	4·75	95·25	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
9 ,,	—	—	—	—	7·77	92·23	13·02	86·98	—	—	—	—	25·8	74·2	21·6	78·4	—	—	—	—	—	—	—	—
10 ,,	4·01	95·99	—	—	—	—	—	—	47·3	52·7	14·92	85·08	—	—	—	—	—	—	—	—	—	—	—	—
11 ,,	—	—	—	—	7·46	92·54	12·7	87·3	—	—	—	—	24·1	75·9	—	—	—	—	—	—	—	—	—	—
12 ,,	—	—	4·13	95·87	—	—	—	—	—	—	—	—	—	—	18·1	81·9	4·27	95·73	38·9	61·1	37·7	62·3	—	—
13 ,,	—	—	—	—	7·4	92·6	12·5	87·5	—	—	—	—	23·4	76·6	—	—	—	—	—	—	—	—	—	—
15 ,,	—	—	—	—	7·3	92·7	12·17	87·83	45·3	54·7	13·45	86·55	22·7	77·3	—	—	3·17	96·83	—	—	—	—	—	—
16 ,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	16·1	83·9	—	—	—	—	—	—	—	—
20 ,,	3·34	96·66	3·73	96·27	—	—	11·8	88·2	44·3	55·7	13·1	86·9	20·3	79·7	15·35	84·65	1·97	98·03	—	—	—	—	—	—
24 ,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	37·6	62·4	36·4	63·6	—	—
25 ,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	14·8	85·2	—	—	—	—	—	—	—	—
30 ,,	2·99	97·01	—	—	6·85	93·15	10·93	89·07	43·3	56·7	11·95	88·05	15·9	84·1	14·43	85·57	1·197	98·803	—	—	—	—	—	—
36 ,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	36·7	63·3	35·6	64·4	36·5	63·5
40 ,,	2·89	97·11	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
48 ,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	36·2	63·8	35·0	65·0	—	—
60 ,,	—	—	—	—	6·71	93·29	9·57	90·43	—	—	—	—	12·55	87·45	12·25	87·75	0·623	99·377	35·7	64·3	34·7	65·3	—	—
80 ,,	2·73	97·27	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
120 ,,	—	—	—	—	6·33	93·67	8·52	91·48	—	—	—	—	10·38	89·62	10·6	89·4	0·278	99·722	—	—	—	—	35·2	64·8
240 ,,	—	—	—	—	6·1	93·9	8·0	92·0	—	—	—	—	8·48	91·52	7·93	92·07	0·166	99·834	—	—	—	—	34·6	65·4
420 ,,	—	—	—	—	—	—	7·5	92·5	—	—	—	—	—	—	—	—	0·098	99·902	—	—	—	—	—	—
480 ,,	—	—	—	—	5·44	94·56	—	—	—	—	—	—	6·0	94·0	4·83	95·17	—	—	—	—	—	—	—	—
12·5 hrs.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	33·3	66·7
24 ,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	31·8	68·2
48 ,,	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	28·3	71·7

NOTE.—In this table the figures for B and C are given as percentages of the total originally present in each experiment.

Conclusions.

1. The establishment of equilibrium in the absorption of agglutinins by their specific bacteria is not attained, as stated by Eisenberg and Volk, in less than five minutes at 0° C., but takes a considerable time, since equilibrium is not reached at room temperature even in four hours.

2. The adsorption of agglutinin or trypsin by charcoal has not reached an equilibrium within four hours at room temperature, and the adsorption of sulphuric acid by charcoal not in 24 hours, or possibly even in 48 hours.

3. There is no justification for judging as to the nature of the interaction between an absorbing material and a substance absorbed, from the rapidity or slowness with which equilibrium is attained, as has been done by Arrhenius.

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On the Absorption of Agglutinin by Bacteria and the Application of Physico-chemical Laws thereto.[*]

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(Communicated by Prof. F. Gotch, F.R.S. Received November 16, 1909,—Read January 20, 1910.)

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Eisenberg and Volk, in 1902, were the first to endeavour to make quantitative measurements of the absorption of agglutinins by bacteria. They showed that if an agglutinating serum in varying dilution was treated with a constant amount of the homologous bacteria, the amount of agglutinin absorbed by the bacteria was not constant. In a concentrated serum the absolute amount absorbed was greater than when the same serum was used after dilution, whilst, on the other hand, the relative amount absorbed from the concentrated serum was less. Hitherto these experiments have been regarded as the fundamental groundwork for the whole discussion on the combination between agglutinins and bacteria.

Arrhenius was the first who tried to apply the laws of physical chemistry to the question of immunity, stating that the interaction between toxins and antitoxins, explained by Ehrlich as complex, was in reality relatively simple. He stated that the combination of a toxin with its antitoxin resembled that of a weak acid—boracic for example—with ammonia, and that the combinations into which the bacterial toxins entered could be explained by the simple laws holding good in the interactions of simple chemical compounds, and without having recourse to the very complicated structures assigned by Ehrlich to diphtheria toxin or tetanus toxin, etc. These theories were mainly based on experiments carried out by Madsen. Arrhenius, from the beginning, has considered the absorption of an agglutinin by its corresponding bacteria as being the most simple one in the domain of immunity, and as being entirely different to the interaction between toxins and their antitoxins.

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[*] The experiments in this paper were partly carried out in the University Laboratory for Medical Bacteriology, Copenhagen, and we wish to express our gratitude to Prof. Salomonsen, the Director of that laboratory, for the great facilities he always granted us while we worked there.

Before describing our own results, we shall briefly mention Arrhenius' view as to the absorption of agglutinins by bacteria. Taking the experiments of Eisenberg and Volk, he showed that there existed a relation between the quantity of absorbed agglutinin (C) and the free agglutinin (B), as expressed by the formula $C = kB^{\frac{2}{3}}$, where k is a constant.

"The physical interpretation of the above formula is very simple. It states that the agglutinin molecules are divided between two solvents, the bacterial cells and the surrounding medium, and that of two molecules of the free agglutinin are formed three molecules of the absorbed agglutinin" (Arrhenius). This is a special case of the Guldberg-Waage law of chemical mass action, and is comparable to the distribution of benzoic acid in the two different solvents, water and benzene, where the concentration of the aqueous solution (C_a) is related to the concentration of the benzene solution (C_b), according to the formula $C_a = kC_b^{\frac{1}{2}}$, as has been shown by Nernst.

Later on, Arrhenius changed his formula from $C = kB^{\frac{2}{3}}$ to $C = kB^n$, as it was found from a series of preliminary experiments carried out by one of us (G. D.), but hitherto not published, that not only the constant k, but also the exponent n varied in different experiments, this variation in the exponent n changing the interpretation of the formula from a simple to an elaborate one.

Arrhenius states in his book on Immuno-Chemistry that n, in the case of the absorption of agglutinin by bacteria, always falls near unity, which is certainly not the case, and he also states that, as n may even be greater than unity, its value has a certain theoretical significance, as an aid in deciding the nature of the process involved in the absorption of the agglutinin.

In support of his theory dealing with equilibria in absorption processes, Arrhenius brings forward the following principal arguments:—

1. That the absorption of agglutinin by bacteria cannot be analogous to the so-called adsorption of dissolved substances by charcoal, or of colouring matter by a fibre (Bordet, Biltz), because the velocity of the reaction in the former case is very great, equilibrium being reached in less than five minutes at 0° C. (Eisenberg and Volk), whereas, in the case of adsorption by charcoal or by a fibre, the process may be incomplete even after several days at the temperature of the room (Bordet, Hedin, etc.).

2. That the absorption of agglutinin by bacteria cannot be a chemical combination in the usual sense, unless it should be a very highly dissociable one, because, even accepting a high degree of dissociation, the fraction of agglutinin fixed (C) should increase to a limit value with increasing

concentration of the amount of agglutinin left (B) in the fluid after absorption has taken place, and with increase of the total amount of agglutinin originally present (T), yet no such limit can be observed in the experiments of Eisenberg and Volk on the absorption of agglutinin by bacteria, or those of Morgenroth and Arrhenius on the absorption of immune body by red corpuscles.

3. That the absorption of agglutinin by bacteria must be in nature different to the so-called adsorption processes, since in the latter n is generally found to be small (*e.g.* in the case of charcoal 0·25, Schmidt), while in the case of bacteria and agglutinin the value of n is from $\frac{2}{3}$ to 1.

4. That the accordance between the observed values and those calculated by the formula $C = kB^n$ (where $n = \frac{2}{3}$) is as good as could be expected in experiments of this kind, owing to the great difficulties in the technique.

Having thus briefly stated the arguments brought forward by Arrhenius in support of his view of the equilibria in the absorption processes of agglutinin by bacteria, we shall go on to our own experiments.

In deciding whether any such given formula offers a correct summarisation of the experimental facts upon which it is based, it is of the greatest importance that the experiments should be very numerous. It is no less important that the experiments should cover a wide range of concentrations. If these precautions are omitted, small deviations from the calculated values will easily be misinterpreted as experimental errors, when in point of fact they are periodical variations occurring with the utmost regularity. Now one of us (G. D.) had proved, in 1904, that the interaction between Coli agglutinin and the filtrate of old Coli culture ("toxin") did not follow the partition law $C = kB^n$ given by Arrhenius, which one might expect it to do if that law actually governed the combination of agglutinin with the specific substance in the bacteria. In addition, it seemed probable from the experiments of Eisenberg and Volk, and Morgenroth and Arrhenius, that periodical variations between values calculated according to this formula and those observed experimentally would occur. Accordingly, our own experiments were designed in such a way and given such a range as to minimise the likelihood that important differences such as these would be missed.

The technique employed was in all detail the same as that already described in our paper on the Velocity of Reaction in the Absorption of Specific Agglutinins by Bacteria, etc.* In the following experiments, coli and typhoid sera of different strengths and age, obtained from various animals immunised with different strains of bacteria, were used. Bacteria or bacterial filtrates ("toxin"), homologous to the serum taken for the experiment, were always used, but in the case of a polyvalent serum only one race of the homologous bacteria was tried, the standardised test emulsion being in every case made of the same strain of bacteria as had been used for absorbing the agglutinins.

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* P. 168, *supra*.

In every experiment, the volume of the bacterial emulsion was kept constant (4 c.c.), and allowed to act on a constant volume (4 c.c.) of the agglutinating serum in various dilutions. If volumes other than these were used, the fact will be found stated in the tables appended to this paper.

After mixing the bacterial emulsion, to be used for absorption, with the various dilutions of serum to be acted on, the tubes were immediately shaken, corked, and placed in a water bath at 37° C. for two hours, being shaken up again at the end of each hour. The tubes were then centrifugalised, and the supernatant fluid tested for the strength of its agglutinin content as described in the previous paper.

In some cases it was of importance to estimate a quantity of agglutinin less than one unit, and this was easily effected by substituting a part of the saline in each tube in the titration by a constant known fraction of a unit of agglutinin, obtained by dilution of the original serum, without altering the total volume in the tube. This method enabled us to determine quantities as small as nearly 0·4 unit. All values of agglutinin are expressed in arbitrary units as explained in the former paper.

Throughout our paper the following terms are used:—

T = the total number of units which the given dilution of serum contained before absorption.

B = the number of units left free in the supernatant fluid after absorption has taken place.

C = T minus B, *i.e.* the number of units removed from the original solution by the absorbing matter.

From the following description of our experiments, it will be clearly seen that our results are absolutely contradictory to the statements of Arrhenius on nearly every point, and we will therefore discuss in the light of our experiments each of the arguments summarised above and brought forward by that gifted chemist and mathematician in support of his theory that the interaction of bacteria and agglutinin can be expressed by the formula $C = kB^n$.

1. In another paper* we have fully proved that in the absorption of agglutinin by bacteria a considerable time elapses before equilibrium is

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* G. Dreyer and J. S. C. Douglas, "The Velocity of Reaction in the 'Absorption' of Specific Agglutinins by Bacteria, and in the 'Adsorption' of Agglutinins, Trypsin, and Sulphuric Acid by Animal Charcoal," *supra*, p. 168.

reached. Thus we cannot accept the statement made by Eisenberg and Volk, and accepted by Arrhenius as being "in good agreement" with his theory, that the reaction has reached equilibrium "in less than five minutes even at 0° C."

2. In view of the statement by Arrhenius, based on the figures of Eisenberg and Volk for the absorption of agglutinin by bacteria and his own figures for the absorption of immune body obtained in conjunction with Morgenroth, that no limit value for C with increasing concentration of B can be observed, the following observations we have made are of great interest.

In Experiments 6, 8, 10, 11, 12 (Tables VII, IX, XI, XII, XIII) it will be seen that there is a great tendency for C to reach a limit value, in spite of an increase in the concentration of T, the total amount of agglutinins originally present; thus in Experiment 8, for example, it is seen that C remains practically the same, although T increases from 1845 to 2882, actually being 1475 and 1477. These experimental observations cannot be brought into agreement with Arrhenius' statement.

That this was not the peculiarity of a single stock of bacteria and its homologous serum is proved by the fact that Experiments 6 and 8 were carried out with a polyvalent serum, Experiment 10 with a different serum, and Experiments 11 and 12 with yet a third variety, as is detailed in the tables.

Further, it can be seen that this phenomenon does not depend on the agglutinating strength of the serum, nor on the amount absorbed, since in Experiment 8 the maximum absorption is 1477 units out of 2882, whilst in Experiment 12 the maximum absorption is only 147 out of the 732.

The point at which this limit value will be reached is dependent, not on the actual amount of agglutinin units present in the serum, but on a number of conditions, such as the amount of bacterial emulsion, the actual dilution of the serum, etc. Thus, if for instance a limit value is reached at a given concentration of the serum by treating it with a number of bacilli 10 x, the limit value would be reached at a lower concentration if the serum was treated with only x, and at a higher one if with 100 x, as is demonstrated by Experiments 6 and 8, and again in Experiments 11 and 12.

Therefore one of the main pillars in support of Arrhenius' theory on the absorption of agglutinins by bacteria falls to the ground because a continued increase in the size of C is an absolute necessity for the application of his formula $C = kB^n$, and thus for his explanation of the nature of the phenomenon.

Passing now to Experiments 2, 5, 7, 9, 14 (Tables III, VI, VIII, X, XV), we see that with a constant amount of bacteria and an increase in the total amount of agglutinin present, C not only reaches a limit value at a certain concentration, but after this point has been reached actually decreases whilst the total amount of agglutinin present further increases, until at a given concentration no diminution in the agglutinin content of the serum can be traced as a result of its treatment with the bacterial emulsion; indeed, there seems in some cases even to be a tendency to a slight increase in the agglutinating strength of the serum, though not, in this series of experiments, of such degree as to be beyond the range of experimental error.

It is difficult to say how this phenomenon is to be explained, but, that it cannot be a question of the presence of so-called "agglutinoids" (Eisenberg and Volk) in the serum, the existence of which had been rendered most unlikely by the previous experiments of Dreyer and Jex-Blake, is clearly seen from the following fact.

If a serum is first treated with great quantities of bacteria it is found by afterwards using it for absorption experiments that not only is the attainment of a limit value not prevented, but that even the decrease in the actual size of C with increasing concentration of T is still evident (Experiment 9, Table X). This, however, could not occur if, as is said to be the case, the agglutinoids have a greater avidity for bacteria than the agglutinins have. A previous treatment of the serum by bacteria would free it from all, or the greater part of, such agglutinoids, so that the limiting value for C found after they have presumably been greatly diminished or got rid of cannot in any way be attributed to them.

The correctness of the statement that this phenomenon is not due to "agglutinoids" will be further proved by the absorption experiments undertaken with fresh agglutinating serum in both a heated and an unheated condition, referred to later in this paper.

In our opinion the phenomenon of a decrease in the value of C with increasing concentration of the serum is most likely caused by some obscure alterations in the surface tension due to change in the concentration of the albumen or of the different salts, or in the viscosity of the fluid, etc.

Such a limit value in C, and even an actual decrease in the size of C, may be reached, not only in the absorption processes of agglutinin by their own homologous bacteria, but may also occur if the agglutinin is acted on by non-specific bacteria, as, for example, a Coli agglutinin by an emulsion of typhoid bacilli (see Experiment 2, Table III).

We have also obtained similar results by treating an agglutinin in various dilutions with constant amount of animal charcoal, as will be described in full in a later paper dealing with that subject,* and in addition we have been able to prove from Bayliss' figures dealing with the adsorption of Congo red by filter paper that the adsorbed amount C not only reaches a maximum, but even decreases subsequently, a fact to which Bayliss himself has drawn no attention.

3. That Arrhenius has no justification for drawing conclusions as to the nature of the interaction between bacilli and the homologous agglutinin from the size of the exponent n in his equation $C = kB^n$, a point on which he lays great stress as a means of distinguishing it from the adsorption processes, is clearly shown by our experiments.

Before dealing with the size of n and k it is worth while mentioning that if this formula were applicable, the line obtained by plotting out the logarithms of the values of B as ordinates, and of C as abscissæ, would be a straight one. How far this is from being the case can be seen from every single experiment recorded in this paper, and is further evident from the curve herein published. By plotting out the logarithms of the values of B and C in this way it is possible to determine n and k.

From Experiment 14 and curve, fig. 1, where the logarithms for values of

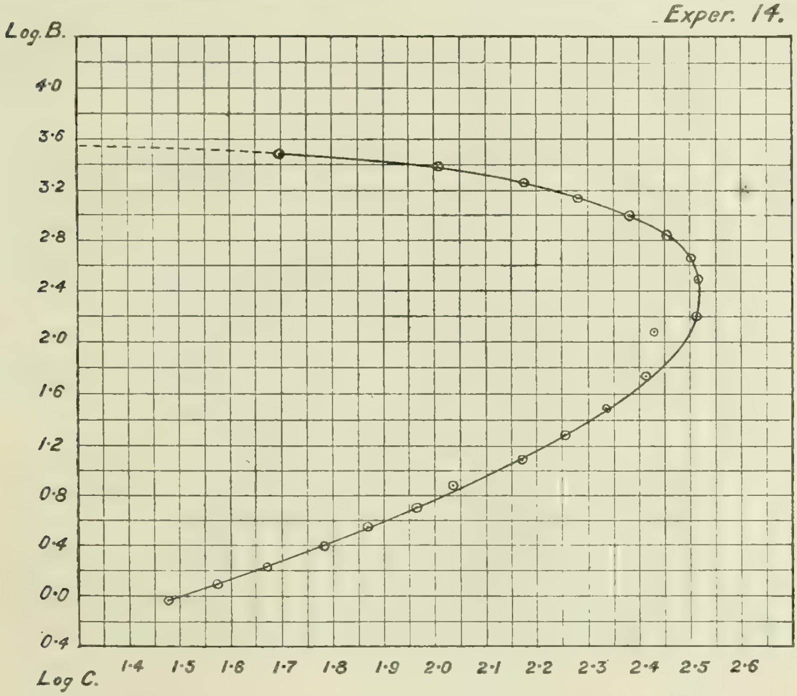

Fig. 1.

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* A preliminary communication on this subject was given at the January meeting the Pathological Society of Great Britain and Ireland, 1909.

Table I.

	Experiment 7.						Experiment 14.				
	T.	B.	C.	"n" (when k = 15·74).	"k" (when n = 0·8834).		T.	B.	C.	"n" (when k = 32·0).	"k" (when n = 0·7161).
1 ...	2750	2750	0	—	—	1 ...	4850	4850	0	—	—
2 ...	2200	2170	30	0·0814	0·0339	2 ...	3880	3880	0	—	—
3 ...	1760	1600	160	0·3143	0·2363	3 ...	3100	3050	50	0·0556	0·1599
4 ...	1403	1140	263	0·4000	0·5242	4 ...	2475	2373	102	0·1491	0·3904
5 ...	1128	795	333	0·4569	0·9124	5 ...	1990	1840	150	0·2054	0·6990
6 ...	907·5	556	351·5	0·4913	1·321	6 ...	1600	1410	190	0·2456	1·0560
7 ...	715	354	361	0·5338	2·021	7 ...	1260	1020	240	0·2908	1·682
8 ...	550	196	354	0·5903	3·357	8 ...	970	684	286	0·3355	2·668
9 ...	440	108	332	0·6511	5·307	9 ...	776	457	319	0·3754	3·972
10 ...	357·5	60·5	297	0·7160	7·921	10 ...	631	303	328	0·4073	5·483
11 ...	275	34·4	240·6	0·7707	10·56	11 ...	485	160	325	0·4567	8·580
12 ...	220	22·8	197·2	0·8084	12·46	12 ...	388	118	270	0·4470	8·865
13 ...	176	16·5	159·5	0·8260	13·41	13 ...	310	50·6	259·4	0·5332	15·62
14 ...	140·3	11·8	128·5	0·8506	14·52	14 ...	247·5	31	216·5	0·5567	18·51
15 ...	112·8	9	103·8	0·8584	14·90	15 ...	199	18·9	180·1	0·5877	21·95
16 ...	90·75	6·7	84·05	0·8806	15·69	16 ...	160	11·8	148·2	0·6210	25·31
17 ...	71·5	5·1	66·4	0·8834	15·74	17 ...	126	7·7	118·3	0·6404	27·43
18 ...	55	3·8	51·2	0·8834	15·74	18 ...	97	5	92	0·6560	29·05
						19 ...	77·6	3·6	74	0·6543	29·56
						20 ...	63·1	2·5	60·6	0·6969	31·45
						21 ...	48·5	1·7	46·8	0·7161	32·00
						22 ...	38·8	1·25	37·55	0·7161	32·00
						23 ...	31	0·95	30·05	1·2286	31·18

B and C have been plotted out, it is clearly seen that it is quite impossible to draw a straight line through more than two consecutive points, but that the line drawn through all the points is actually a curve deviating more and more from the straight line, and away from the abscissa as the concentration of the agglutinating serum is increasing. This is caused by the fact that from the very outset C does not increase as rapidly as it should according to Arrhenius' formula. Also it is seen from this and the other experiments how impossible it is to find a constant exponent n if k has to be a constant, or *vice versâ*, as demanded by Arrhenius' formula $C = kB^n$. This is well shown in the following table of figures calculated from Experiments 7 and 14 (Table I).

On the other hand, it is possible in experiments which only cover a short range, to draw a line which will allow the determination of an n and k which will give calculated values for B approximating to those observed—but with a regular periodicity in the deviation.

A similar periodicity in the change of value of k is also to be traced in the experiments of Eisenberg and Volk, and of Arrhenius and Morgenroth, though with some difficulty owing to the great distance between the points experimentally determined.

In the different tables (II to XV) we have calculated from point to point the exponent n, and it exhibits enormous variations in size according to the region examined in each experiment.

In Experiment 14 (Table XV), for example, we find that n will have a value of about 0·8 in the weakest concentration of the serum. From this point it will gradually decrease in size to zero, and then, becoming negative in sign, increase towards infinity. That this behaviour does not depend on the strain of bacillus or the brand of agglutinating serum is proved by any or all of the experiments undertaken with various bacteria and their homologous agglutinin (see Experiments 1, 2, and 5 to 14, Tables II, III, and VI to XV).

Experiment 5 (Table VI) proves that the variation in n is not caused by the presence of the quantities of formalin added to the emulsions.

To make it clear that this deviation could not be explained by the presence of "agglutinoids" in the serum, or substances analogous to such bodies in the bacteria, experiments which gave absolutely similar results were undertaken with heated serum and unheated Coli bacilli filtrate ("toxin") (Experiments 3 and 4, Tables IV and V), and with heated bacilli and unheated serum (Experiments 1 and 6, Tables II and VII). Now, if any such bodies had been formed by the action of heat, the deviation from a straight line of the curve obtained by plotting out log. B and log. C should become more marked. That this, however, is not the case, is readily seen.

Experiment 7 (Table VIII), in which the serum before use had been treated with bacteria to remove such bodies if they were present, offers additional proof on this point.

It is very interesting to note that it is not at present possible to distinguish between the action of the Coli bacilli filtrate ("toxin") and that of the bacteria themselves, on agglutinin; this is seen from Experiments 2 and 3 (Tables III and IV). Therefore the supposition expressed by one of us (G. D.) on an earlier occasion that, as the partition law of Arrhenius did not hold good for the interaction of such "toxin" and agglutinin the same would most likely be found to be the case if the interaction of bacteria and agglutinin were examined, was correct.

A further point of interest is that in spite of the partition law not holding good in the case of "toxin" and agglutinin, the above formula will, nevertheless, if used for calculation, give a better agreement between calculated and experimental figures in this case than in that of bacteria and agglutinin.

Comparing together the absorptions of agglutinin from heated serum and from unheated serum, and plotting out the values log. B and log. C, it appears that the deviation from the straight line is less in the case of the heated serum. The higher the temperature, within certain ranges, to which the serum is heated, the more constant will n be found within corresponding ranges of concentration of serum, and the nearer to a straight line will be the curve obtained. Thus in Experiment 4 (Table V), where the serum was heated to 70° C., and there was an increase in concentration from about 4 to 60 units, nothing better than a straight line can be drawn, giving an n of about 0·73, and a k of about 1·0, while in Experiment 3 (Table IV), where the serum is only heated to 60° C., n varies from about 1·1 to 0·3.

From the whole series of experiments it is clear that one is not justified from the size of n in forming a conclusion (as done by Arrhenius) as to the nature of the interaction, because n may have any size from near one to minus infinity in the same experiment, depending only on the concentration of the serum. This is the more the case since we have found variations in the size of n of very much the same kind in the "adsorption" of agglutinins and other bodies by charcoal, as we shall describe in a later paper.*

It is further seen from Experiments 1 to 14 (Tables II to XV) that no great stress can be laid on the actual size of n or k, since n is not alone, or even mainly, dependent upon the kind of absorbing matter or substance absorbed, but on the varying amounts of both, the presence of albumen, dilution of serum, presence of salts, temperature, time of reaction, and other variable factors.

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* A preliminary communication on this subject was given at the January meeting of the Pathological Society of Great Britain and Ireland, 1909.

4. Turning to the fourth point brought forward by Arrhenius, that the agreement is satisfactory between observed figures and those calculated according to his formula $C = kB^n$ and published in his papers, we consider that the accordance between his figures is by no means good. Arrhenius himself regards the deviations he finds as entirely due to experimental error, because he was informed by Eisenberg and Volk that such great variations were quite possible in the technique that they had used. To us it is clear that, leaving alone the great experimental error, all the experiments calculated by Arrhenius show a certain periodicity in the increase and decrease of k in the same direction as found in our own experiments, but not taken into consideration by him. Our own results show that, even if we chose the best possible n, B calculated and B observed will only agree approximately within a certain small range. Beyond these limits the values for B observed will be much bigger than for B calculated, a natural result of what has been stated previously, that C does not increase so fast as it should if Arrhenius' formula $C = kB^n$ were correct.

As we have proved beyond dispute the great and regular variations occurring in the "constants" n and k of Arrhenius' formula $C = kB^n$ when applied to the absorption of agglutinin by bacteria, we record no figures calculated according to that expression, since it is now obvious how absolute must be the disagreement between the observed values of B and those thus calculated.

At a later date it is our hope to deal with the mathematical considerations arising in connection with the figures in this paper.

Table II.—Experiment 1. October 26, 1904.

5 c.c. of typhoid serum (T 29). 5 c.c. of 1·5/1 N emulsion of typhoid bacilli without formalin. Y, bacilli unheated; Z, bacilli previously heated to 60° C. for 1 hour. Actual strength of serum in tube 1 = 50 per cent.

		Y.			Z.		
	T.	B.	C.	*n.*	B.	C.	*n.*
1	111·2	76·9	34·3	0·186	78·0	33·2	0·297
2	44·4	18·2	26·2	0·57	21·7	22·7	0·704
3	22·2	7·0	15·2	0·876	9·5	12·7	1·004
4	11·1	3·22	7·88	0·798	4·76	6·34	1·31
5	5·55	1·43	4·1		2·63	2·92	0·870
6	2·78	<1·0	—		1·25	1·53	
7	1·39	<1·0	—		<1·0	—	

Table III.—Experiment 2. November 10, 1904.

5 c.c. *Coli lab.* serum (*x*) 61, 1/1. Y, 10/1 N *Coli lab.* bacilli without formalin. Z, 10/1 N typhoid bacilli without formalin. Actual strength of serum in tube 1 = 50 per cent.

		Y.			Z.		
	T.	B.	C.	*n.*	B.	C.	*n.*
1	2000	1970	30	2·16	2000	0	—
2	1000	775	225	0·154	911	89	0·28
3	500	305	195	0·435	428	72	0·737
4	250	120	130	0·571	211	39	0·956
5	125	48	77	0·508	105	20	0·839
6	62·5	17	45·5	0·551	51·5	11·0	0·881
7	31·3	5·9	25·4		25·4	5·9	0·91
8	15·6	1·0	—		12·5	3·1	0·91
9	7·8	1·0	—		6·17	1·63	

Table IV.—Experiment 3. February 9, 1905.

1 c.c. of Coli serum (x) 61, unheated (Y) and heated undiluted to 60° C. for 1 hour (Z). 9 c.c. Coli bouillon culture filtrate ("Toxin" 21), grown 21 days at 37° C. before filtration. Actual strength of serum in tube 1 = 10 per cent.

	Y.				Z.			
	T.	B.	C.	*n*.	T.	B.	C.	*n*.
1	880	775	105	0·151	708	490	218	0·535
2	440	347	93	0·213	354	214	140	0·569
3	220	143	77	0·303	177	91	86	0·57
4	110	53	57	0·517	88·5	37	51·5	0·651
5	55	20·3	34·7	0·865	44·3	15·3	29	0·894
6	27·5	9·5	18	1·03	22·1	7·24	14·86	1·255
7	13·8	4·76	9·04	1·11	11·06	4·0	7·06	0·895
8	6·9	2·46	4·44	1·17	5·53	1·9	3·63	0·995
9	3·4	1·3	2·1		2·77	0·95	1·82	

Table V.—Experiment 4. February 13, 1905.

1 c.c. of Coli serum (x) 61, diluted 1/5 and then heated to 70° C. for 1 hour. 9 c.c. of Coli ("Toxin" 21) (see Exp. 3, Table IV). Actual strength of serum in tube 1 = 2 per cent.

	T.	B.	C.	*n*.
1	60·5	41·7	18·8	0·789
2	30·25	19·8	10·45	0·738
3	15·13	9·2	5·93	0·725
4	7·56	4·2	3·36	0·732
5	3·78	1·9	1·88	
6	1·89	<1·0	—	

Table VI.—Experiment 5. February 18, 1905.

5 c.c. of Coli serum (x) 61, diluted 1/2. 5 c.c. of 10/1 N Coli culture without formalin. Actual strength of serum in tube 1 = 25 per cent.

	T.	B.	C.	*n*.
1	1820	1820	0	
2	910	810	100	−0·24
3	455	331	124	0·121
4	227·5	118	109·5	0·205
5	113·8	30·7	83·1	0·215
6	56·9	3·82	53·08	
7	28·4	<1·0		
8	14·2	<1·0		

Table VII.—Experiment 6. March 12, 1907.

4 c.c. Coli serum, Forsög's polyvalent, diluted 1/5. 4 c.c. 10/6 N emulsion of Coli Aunsögaard, February 23, 1907. Y, unheated; Z, previously heated to 100° C. for 5 minutes. Actual strength of serum in tube 1 = 10 per cent.

		Y.			Z.		
	T.	B.	C.	*n*.	B.	C.	*n*.
1	530	159	371	0·0768	162	368	0·0734
2	432	80	352	0·37	82	350	0·397
3	349	51	298	0·567	53·5	295·5	0·750
4	283	36·5	246·5	0·595	41	242	0·923
5	231	27·5	203·5	1·17	33·0	198·0	0·95
6	188	23	165	0·725	26·6	161·4	1·083
7	153·5	17·6	135·9	0·905	22	131·5	1·048
8	124·5	14	110·5	0·594	18	106·5	0·641
9	100·5	10	90·5		13·2	87·3	

Table VIII.—Experiment 7. March 20, 1907.

4 c.c. of Coli serum, Forsög's polyvalent, 1/1. 4 c.c. of 10/9 N Coli Aunsögaard emulsion, February 23, 1907. Actual strength of serum in tube 1 = 50 per cent.

	T.	B.	C.	*n.*
1.........	2750	2750	0	
2.........	2200	2170	30	
				−5·49
3.........	1760	1600	160	
				−1·467
4.........	1403	1140	263	
				−0·649
5.........	1128	795	333	
				−0·145
6.........	907·5	556	351·5	
				−0·0591
7.........	715	354	361	
				0·0328
8.........	550	196	354	
				0·109
9.........	440	108	332	
				0·192
10.........	357·5	60·5	297	
				0·374
11.........	275	34·4	240·6	
				0·478
12.........	220	22·8	197·2	
				0·656
13.........	176	16·5	159·5	
				0·646
14.........	140·3	11·8	128·5	
				0·787
15.........	112·8	9·0	103·8	
				0·715
16.........	90·75	6·7	84·05	
				0·864
17.........	71·5	5·1	66·4	
				0·883
18.........	55	3·8	51·2	

Table IX.—Experiment 8. March 22, 1907.

4 c.c. of Coli serum, Forsög's polyvalent, 1/1. 4 c.c. of 10/2 N Coli Aunsögaard emulsion, March 15, 1907. Actual strength of serum in tube 1 = 50 per cent.

	T.	B.	C.	*n*.
1 ………	2882	1405	1477	0·00555
2 ………	1845	370	1475	0·372
3 ………	1469	231	1238	0·680
4 ………	951	127	824	0·831
5 ………	749	96	653	0·957
6 ………	461	58	403	

Table X.—Experiment 9. April 12, 1907.

4 c.c. of an old *Coli lab.* serum (goat), previously diluted and treated with an emulsion of bacilli. 4 c.c. of 10 N *Coli lab.* emulsion, April 8, 1907. Actual strength of serum in tube 1 not known.

	T.	B.	C.	*n*.
1 ………	411	222	189	−0·0826
2 ………	339	143	196	0·0106
3 ………	253	58·8	194·2	0·133
4 ………	209	30·8	178·2	0·224
5 ………	168	15·4	152·6	0·296
6 ………	136	8·4	127·6	0·351
7 ………	107	4·5	102·5	0·461
8 ………	82·2	2·6	79·6	0·509
9 ………	65·8	1·7	64·1	
10 ………	53·4	<1·0		
11 ………	41·1	<1·0		
12 ………	33·9	<1·0		

Table XI.—Experiment 10. April 26, 1907.

4 c.c. of Coli Kringelgaard (horse) serum, 1/1. 4 c.c. of 20 N Coli Kringelgaard emulsion, April 24, 1907. Actual strength of serum in tube 1 = 50 per cent.

	T.	B.	C.	*n.*
1	961	363	598	0·0349
2	768	184	584	0·0402
3	615	67	548	0·136
4	490	21·3	468·7	0·204
5	394	8·3	386·7	0·29
6	317	4·0	313	
7	250	>2	248	0·348 (from Nos. 6 and 8)
8	194·5	1·0	193·5	
9	153·8	<1·0		
10	124·9	<1·0		

Table XII.—Experiment 11. October 14, 1908.

4 c.c. of *Coli lab.* (goat) serum, June 16, 1907, 1/1. 4 c.c. of 30 N *Coli lab.* emulsion, June 26, 1907. Actual strength in tube 1 = 50 per cent.

	T.	B.	C.	*n.*
1.........	805	209	596	0·235
2.........	643	120	523	0·215
3.........	515	61·7	453·3	0·222
4.........	411	28·7	382·3	0·274
5.........	330	14·3	315·7	0·324
6.........	266	7·7	258·3	0·592
7.........	209	4·76	194·24	0·482
8.........	161	3·1	157·9	0·71
9.........	128·8	2·27	126·53	0·895
10.........	104·6	1·8	102·8	0·801
11.........	80·5	1·3	79·2	0·666
12.........	64·3	0·93	63·37	0·866
13.........	51·5	0·72	50·78	0·904
14.........	41·1	0·55	40·55	0·9
15.........	33	0·44	32·56	
16.........	26·6	<0·31		
17.........	20·9	<0·31		
18.........	16·1	<0·31		

Table XIII.—Experiment 12. October 19, 1908.

4 c.c. of *Coli lab.* (goat) serum, June 16, 1907, 1/1. 4 c.c. of 6·67/1 N *Coli lab.* emulsion, June 26, 1907. Actual strength of serum in tube 1 = 50 per cent.

	T.	B.	C.	*n*.
1.........	723	585	147	0·125
2.........	586	444	142	0·122
3.........	468	331	137	0·131
4.........	373·5	242	131·5	0·149
5.........	300·3	175	125·3	0·131
6.........	241·5	122	119·5	0·146
7.........	193	80·5	112·5	0·190
8.........	146·3	45·4	100·9	0·183
9.........	117·1	26	91·1	0·301
10.........	95·2	16·2	79·0	0·246
11.........	73·2	7·6	65·6	0·272
12.........	58·6	3·9	54·7	0·5
13.........	37·35	1·66	35·69	0·885
14.........	30·03	1·3	28·73	0·615
15.........	24·15	0·92	23·23	0·696
16.........	19·3	0·67	18·63	0·283
17.........	16·63	0·41	16·22	

Table XIV.—Experiment 13. December 17, 1908.

4 c.c. of *Coli lab.* (goat) serum, June 16, 1907, 13/100. 4 c.c. of 12·5/1 N *Coli lab.* emulsion. Actual strength of serum in tube 1 = 6·5 per cent.

	T.	B.	C.	*n*.
1	68·8	8·33	60·47	0·302
2	53·0	4·12	48·88	0·402
3	42·4	2·49	39·91	0·625
4	33·9	1·76	32·14	0·848
5	27·0	1·35	25·65	0·898
6	21·1	1·05	20·05	1·049
7	17·5	0·83	16·67	

Table XV.—Experiment 14. June 14, 1909.

4 c.c. of *Coli lab.* (goat) serum, February 26, 1909, 1/1. 4 c.c. of 40 N *Coli lab.* emulsion, May 29, 1909. Actual strength of serum in tube 1 = 50 per cent.

	T.	B.	C.	*n*.
1	4850	4850	0	
2	3880	3880	0	
3	3100	3050	50	
				−2·84
4	2475	2373	102	
				−1·52
5	1990	1840	150	
				−0·889
6	1600	1410	190	
				−0·722
7	1260	1020	240	
				−0·466
8	970	684	286	
				−0·2710
9	776	457	319	
				−0·0677
10	631	303	328	
				0·0144
11	485	160	325	
				0·196 (from Nos. 11 to 13)
12	388	118	270	
13	310	50·6	259·4	
				0·368
14	247·5	31	216·5	
				0·372
15	199	18·9	180·1	
				0·413
16	160	11·8	148·2	
				0·528
17	126	7·7	118·3	
				0·583
18	97	5·0	92·0	
				0·662
19	77·6	3·6	74·0	
				0·548
20	63·1	2·5	60·6	
				0·671
21	48·5	1·7	46·8	
				0·716
22	38·8	1·25	37·55	
				0·811
23	31·0	0·95	30·05	
24	24·75	<1·0		
25	19·9	<1·0		
26	16·0	<1·0		
27	12·6	<1·0		

Conclusions.

1. When an agglutinating serum in different concentrations is treated with constant amounts of bacteria, the quantity absorbed C may not

only increase to a limit value but may, when this point is passed, even decrease to zero when the concentration of the serum is further increased.

2. It is impossible, from the greater or smaller size of the exponent "n" in the formula $C = kB^n$, to determine whether in the case of agglutinin we have to deal with an absorption or an adsorption process, as in both cases "n" may vary within nearly the same ranges.

3. The formula $C = kB^n$, proposed by Arrhenius to express the absorption of agglutinin by bacteria, as being a special example of the Guldberg and Waage law of chemical mass action, does not hold good either in the case of absorption of agglutinin by bacteria, or of the neutralisation of agglutinin by homologous bacterial filtrate ("toxin").

4. The combination of agglutinin and bacteria is, therefore, not such a simple process as anticipated by Arrhenius, but is very possibly complex, and not improbably of the same nature as the interaction of bacterial toxins and antitoxins.

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Observations on the Rate of Action of Drugs (Alcohol, Chloroform, Quinine, Aconitine) upon Muscle as a Function of Temperature.

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(Received November 23, 1909,—Read January 20, 1910.)

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Very scanty information exists as regards the effect of temperature upon the activity of drugs. Brunton[*] says :—"That the action of veratrine and of barium on muscle is very much altered by heat and cold. Many, if not all, muscular poisons act more quickly with increased temperature."

According to the same author, Humboldt[†] noticed that warmth increases the rapidity with which alcohol destroyed the irritability of a nerve and potassium sulphide that of a muscle. Brunton and Cash[‡] showed that "up to a certain point heat increases the effect of veratria and cold lessens it." Waller took observations of the rates of action of alcohol, ether, and

[*] 'Pharmacology,' 1893, p. 45.

[†] 'Ueber die gereizte Muskel und Nervenfaser,' 1797, vol. 2, p. 218.

[‡] 'Journ. of Physiol.,' 1883, vol. 4, p. 1.

chloroform at the temperatures 19° and 30°, and gives for ether a coefficient of 2·0 for a rise of 10°.*

None of these observations have been definitely addressed to the question whether or no the pharmacodynamic action of drugs obeys the laws of chemical action in its relation to temperature, although obviously they point in that direction.

The following observations have been made as carefully as possible by methods already described in previous communications. In preliminary experiments we determined the most convenient concentrations to afford us a sufficient number of observations at different temperatures within the range of 7° to 27°. In all cases the effects were markedly increased by rise of temperature; the degree of correspondence will be evident from the details of observations given below. Within the range of 7° to 27° the temperature coefficient of increase in, *e.g.*, the case of quinine (*vide infra*), comes out as 2·52 per 10°. An upper limit is fixed by the heat coagulation of the muscular substance. The lower limit appears to be that at which chemical combination of this peculiar character is suspended. It varies for different drugs, *e.g.*, in the case of the powerful drug aconitine the poisonous effect is completely inhibited at a temperature of about 7°. As will be shown below, this inhibition is temporary.

The question whether the acceleration and retardation of a toxic effect by higher or lower temperature are really of a chemical nature, and not merely by reason of differences of physical diffusion, is very easily answered.

Taking a strength of drug and a low temperature such that chemical action (but not diffusion) is practically suppressed, and subsequently placing in normal saline the muscle in which diffusion (but not combination) of the drug has taken place, we now find the characteristic effect of the drug to manifest itself, *e.g.*, No. 631 (fig. 16). And apparently the rate of this *delayed* effect is not sensibly different from the rate of an undelayed effect, which goes to show that in an ordinary experiment where rate of diffusion and rate of chemical change are to be thought of, the former is negligible in comparison with the latter.

As a preliminary to the study of the effect of temperature upon the rate of action of drugs upon muscle, we naturally took observations of the effect of variations of temperature upon contraction in the absence of drugs. This brought out one very definite and, we believe, important point.

As is well known, contractility subsists between an upper and lower limit of temperature, and it is usually considered that the upper limit is set by the point at which heat coagulation takes place. But the matter is not quite so simple. We find that with rising temperature the contractility (as tested by induction shocks, which are the shortest possible stimuli) vanishes before heat contraction appears.

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* "The Comparative Power of Alcohol, Ether, and Chloroform, etc.," 'Roy. Soc. Proc.,' vol. 81, B, p. 545, 1909.

There is, in fact, after a first augmentation, a change of an anti-excitatory or inhibitory character, a true heat paralysis of the *relaxed* muscle before the more profound abolition attributable to heat coagulation.* If the rise of temperature does not go beyond a certain limited point, this heat inhibition is temporarily followed by perfect recovery. Beyond this point, *i.e.* if coagulation has occurred,† there is no recovery of contractility. In the two examples below the temporary effect has occurred with a rise to 34° —the permanent effect after a rise to 40°.‡

As regards the lower limit, we satisfied ourselves that the temperature could be lowered to at least 0° without abolition of contractility, and in our subsequent experiments we did not lower the temperature below 7°, so that in all certainty any retardation observed at 7° was retardation of chemical change as regards the drug and not the simple effect of cold without drug. Thus, *e.g.*, in the experiment of November 12 (fig. 1), we have an undiminished contraction at 0° for at least half an hour; the muscle undrugged does not lose contractility until congelation actually sets in; this occurs at $-0^\circ{\cdot}6$ to -1°.

The variation of chemical change with temperature is most simply expressed by Esson's formula§

$$K_{T_0}/K_{T_1} = (T_1/T_0)^m, \tag{1}$$

which for our purposes becomes

$$L_0/L_1 = (T_1/T_0)^m, \tag{2}$$

or simply

$$\log L_0 - \log L_1 = m(\log T_1 - \log T_0), \tag{3}$$

in which L_0 and L_1 are the lengths of time required for the abolition of

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[*] "The Effect of Heat upon the Electrical State of Living Tissues," 'Roy. Soc. Proc.,' vol. 81, B, p. 303, 1909.

[†] We do not commit ourselves to the statement that heat-coagulation is an entirely irreversible change. On the contrary, we think that slight degree of such change in the living body can be entirely effaced.

[‡] It has been observed that the contractility of a frog's muscle, suspended in saline (0·5 per cent.) solution increases rapidly with rise of temperature from 28° up to 45°, the maximum point. If the muscle be immersed directly in saline solution at 45°, the contraction is instantaneous. Max Verworn, 'Allgemeine Physiologie,' 1897, p. 398.

[§] 'Phil. Trans.,' A, 1895, vol. 186, p. 861. Esson's formula has been shown in other cases to accord better with observed results than does that of Arrhenius—

$$K = K_0 e^{-\left(\frac{T_0-T_1}{T_1-T_0}\right)}$$

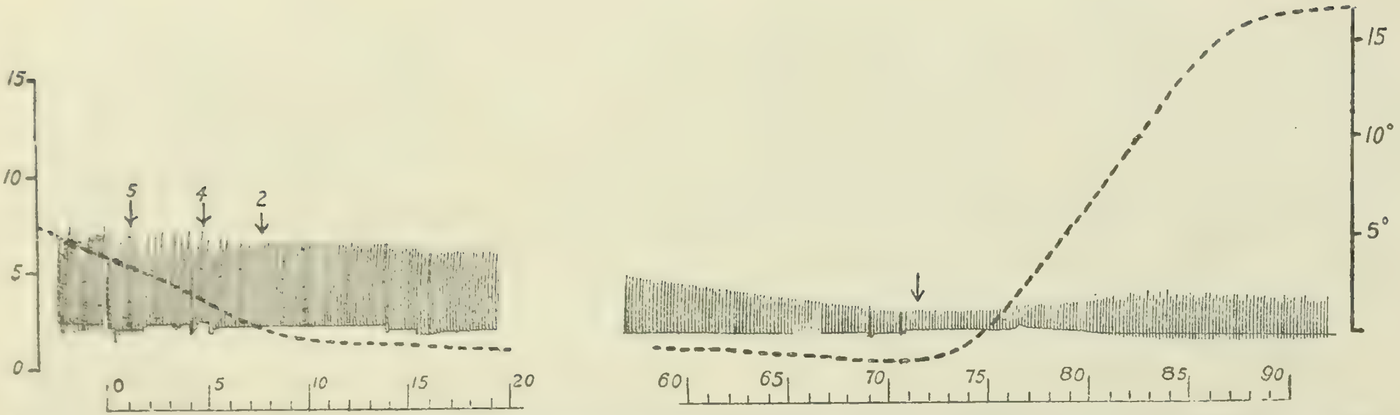

FIG. 1.—November 12, 1909 (679).—Contractions of a muscle immersed in normal saline, the temperature of which is lowered to −1°, then raised to 17°, as indicated by the broken line. (The record between the 20th to the 60th minute is omitted from the figure.)

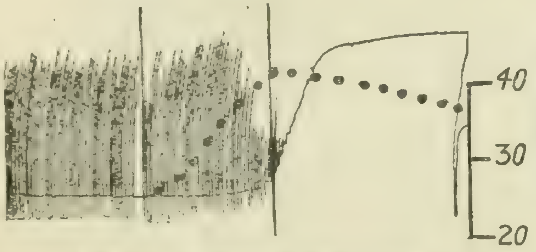

FIG. 2.—Temperature raised to 40°.

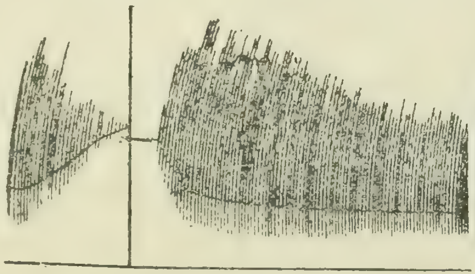

FIG. 3.—Temperature raised to 34°.

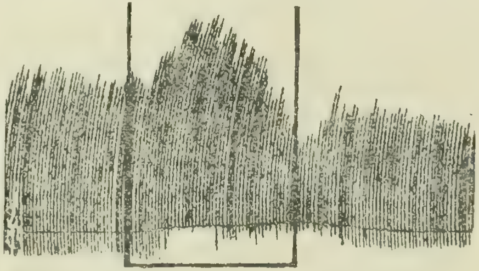

FIG. 4.—Temperature raised to 30°.

FIGS. 2, 3, 4.—Contractions of a muscle immersed in normal saline, the temperature of which is raised to 40°, 34°, and 30° respectively, and then allowed to fall. In the first case (fig. 2) the contraction is permanently abolished with the muscle in heat-rigor. In the second case (fig. 3) the contraction is temporarily abolished, without rigor. In the third case the contraction is temporarily augmented then diminished, without rigor.

contractility under the influence of the drug at the lower and higher temperatures; T_0 and T_1 the respective absolute temperatures. This takes length of time as expressing the rate of chemical change, *i.e.* the rate with which the drug reacts upon the living muscle. The shorter the time, the greater the rate.

Put into words, formula (3) states that the logarithmic increment of chemical change (which in our observations is represented by the logarithmic decrement of time of complete intoxication) varies as the logarithmic increment of absolute temperature; the graph of this relation is a straight line, which affords a convenient means of representing a series of results; for this purpose we have taken time along the abscissa, temperature along the ordinates.

We have selected as typical substances of widely differing toxicities: ethyl alcohol, chloroform, quinine hydrochloride, and aconitine hydrochloride, taken of concentrations such as to bring about the abolition of muscular contraction within convenient lengths of time at temperatures ranging from 7° to 27°. (According to our preliminary observations, the relative toxicities of these substances taken on the molecular scale are: Alcohol = 1, Chloroform = 100, Quinine = 2000, Aconitine = 20,000.)

The accuracy of our time-measurements was always subject to an error of about half a minute by reason of the difficulty of determining the end-point of the abolition of contractility; this error was aggravated in observations taken at low temperatures.

(1) *Ethyl Alcohol.*

Alcohol, sold as "pure absolute," was purified as follows: A stick of sodium hydrate was dissolved in the spirit and the whole heated in a reverse condenser for one hour in order to saponify the esters, and resinify the aldehydes; it was then fractionated, the middle portion, boiling at 78°·9 (corr.), being retained. Two density determinations gave the following results: $S.G.^{15/15} = 0{\cdot}81046$ and 0·81045 respectively, which corresponds to 94·5 per cent. per weight alcohol.

It has been shown in previous investigations that this method of purification removes all impurities other than water, which for our purpose is not material. The alcohol was made up to normal solution: 46 grammes per litre of 0·6-per-cent. saline in tap water.

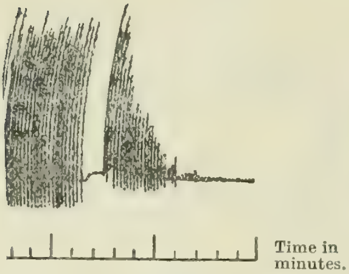

Fig. 5.—Effect of ethyl alcohol, $n/1$, at 24°.

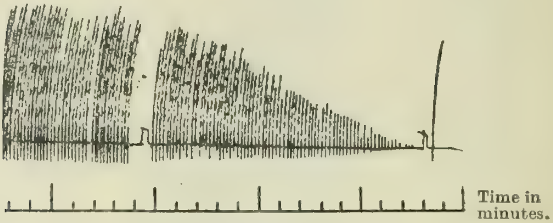

Fig. 6.—Effect of ethyl alcohol, $n/1$, at 17°.

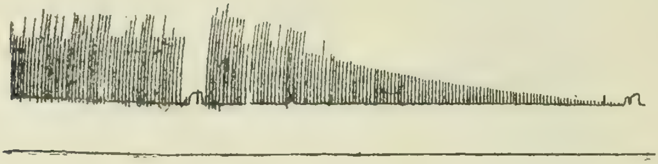

Fig. 7.—Effect of ethyl alcohol, $n/1$, at 7°.

The results are set out in the following table, in which are given the temperatures, the times of abolition taken as the factors of chemical change, their logarithmic differences, and the values for the factor m :—

Ethyl Alcohol.

Temperatures.	Times.	Log T_1/T_0.	Log L_0/L_1.	m.
°				
7	20	0000	0006	
14	12·5	0108	2041	18·9
15	12·0	0121	2218	18·3
17	9·0	0152	3468	22·1
18	9·0	0167	3468	20·7
19	7·5	0182	4259	23·4
20	7·5	0197	4259	21·6
24	6·0	0251	5228	20·5
			Mean	20·8

Note.—The decimal points of the logs. are taken as understood, both in the tables and in the graphs.

The observed and calculated times compare as follows :—

Times (observed).	Times (calculated).	Times (obtained).	Times (calculated).
20	20	9·0	9·0
12·5	11·9	7·5	8·4
12·0	11·2	7·5	7·8
9·0	9·7	6·0	5·9

With one exception, the difference between the observed and calculated times is about 0·5 minute, which is within the probable experimental error.

It may be observed that the mean value of m (20·8) corresponds closely with the value 20·38 observed by Harcourt and Esson for the reaction between hydrogen peroxide and hydrogen iodide. The departure from the mean value of m is $\pm 2{\cdot}5$ in our experiments, which is hardly an excessive variation where the reaction concerns a living tissue. The graph of these results is as under. From the mean value of $m = 20{\cdot}8$ the coefficient of increase for 10° is 2·04.

Graph 1.

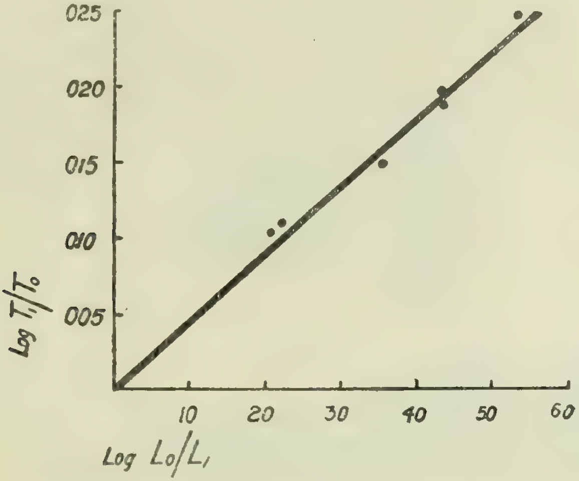

Fig. 8.—Alcohol, $n/1$.

Chloroform.

Chloroform, obtained from chloral hydrate, was weighed out in the quantity required (1·195 grammes per 1000 c.c.) to make a solution of $n/100$ concentration in physiological saline (omitting correction for density). We took this as being the strength physiologically equivalent to that of a molecular solution of alcohol.* The results were set out in the following table:—

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* 'Roy. Soc. Proc.,' B, June 24, 1909.

Chloroform.

Temperatures.	Times.	Log T_0/T_1.	Log L_1/L_0.	m.
°				
7	24·5	0000	0000	
10	21	0047	0670	14·3
19	13	0182	2753	15·1
24	10·5	0257	3650	14·3
24	11	0257	3478	13·5
			Mean ……	14·3

The graph of these results is as under. From the mean value of $m = 14$ the coefficient increase for 10° comes out = 1·63.

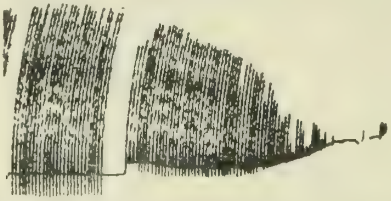

Fig. 9.—Effect of chloroform, $n/100$, at 24°.

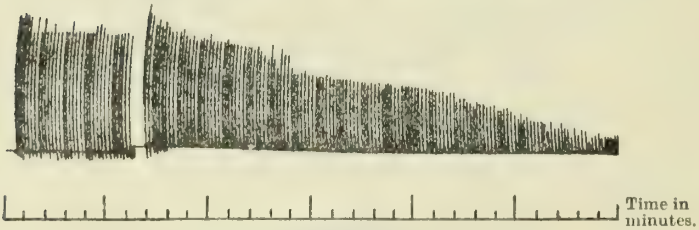

Fig. 10.—Chloroform, $n/100$, at 10°.

Graph 2.

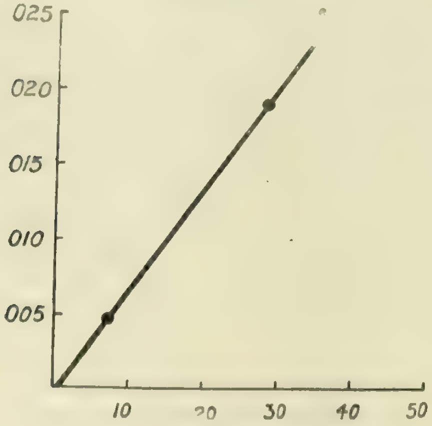

Fig. 11.—Chloroform, $n/100$.

The observed and calculated times compare as follows :—

Times (obs.).	Times (calc.).
24·5	24·5
21	21
13	13·5
10·5	10·5
11·0	10·5

The error is ±0·5 minute.

Quinine Hydrochloride.

We took quinine hydrochloride as a typical and familiar drug readily obtainable in reasonable purity, and with the action of which upon muscle we had become well acquainted in course of comparative observations on the cinchona alkaloids.* The results were as follows :—

Temperature.	Times.	Log T_1/T_0.	Log L_0/L_1.	*m*.
°	mins.			
7	53	0·0000	0·0000	
12	35	0·0076	0·1802	26
18	22	0·0167	0·3818	22·8
19	16	0·0182	0·5201	28·5
19	15·5	0·0182	0·5339	28·8
20	15	0·0197	0·5481	28·3
25	10·5	0·0270	0·7030	26·0
			Mean	26·7

The graph of these results is as under. From the mean value of $m = 26·7$ the coefficient of increase for 10° is 2·52, a number which is not widely different from that found (viz., 2·44) for the reaction between chloric acid and ferrous sulphate.†

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* 'Physiol. Soc. Proc.,' Oct., 1909.

† Hood, 'Phil. Mag.,' 1885, [5], vol. 24.

Quinine.

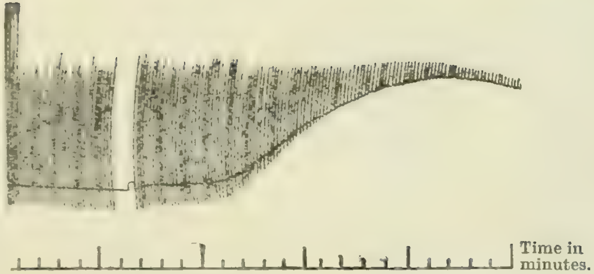
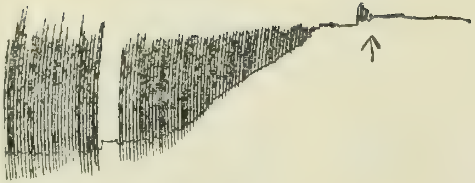


Fig. 12.—Effect of quinine hydrochloride, $n/2000$, at 19°.



IG. 13.—Effect of quinine hydrochloride, $n/2000$, on muscular contraction at 25°.

Quinine HCl, $n/2000$.

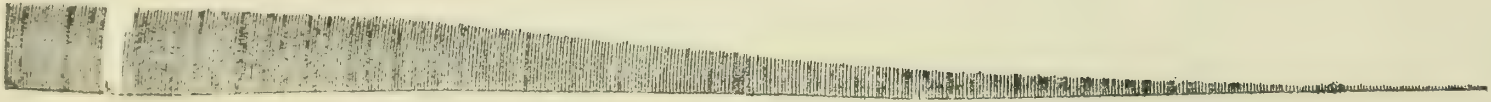


Fig. 14.—Effect of quinine, $n/2000$, on muscular contraction, at 7°.

Graph 3.

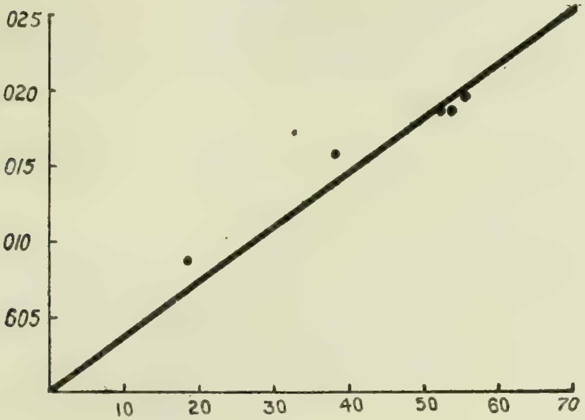


FIG. 15.—Quinine, $n/2000$.

The observed and calculated times compare as under :—

Times (obs.).	Times (calc.).
53	53
35	33·8
22	19
16	17·4
15·5	17·4
15	15·8
10·5	11·2

The concordance is not so good as in the two preceding series; this is to be expected, partly from the high temperature coefficient and partly on account of the difficulty of ascertaining the end-point.

Aconitine Hydrochloride.

We selected aconitine as being the most powerful poison known to us, acting upon muscle at a dilution reaching $n/100000$. In our first trials at strengths of $n/1000$ and $n/2000$ we found that it was necessary to cleanse the vessels with great care, otherwise an evident aconitine effect might present itself as a fallacy.

The effect of low temperature was particularly striking. The toxic action of solutions $n/10000$, and even of $n/2000$ and $n/1000$, was completely suspended, while the temperature of solution, and presumably of muscle, was at 7° to 8°, but made its appearance as soon as the temperature was raised.

We have already commented on this remarkable result as affording evidence of the chemical factor as distinct from the diffusion factor.

The results in this respect were as follows :—

Plate.	Concentration.	Temperature.	Time.	Temperature.	Time.
		°		°	mins.
634	*n* 0·0001	7	∝	18	25
681	0·0005	7	∝	19	6·5
682	0·0010	7	∝	19	3·5

We incidentally observed the course of recovery of muscles when the experimental solution was replaced by normal saline. After alcohol, chloroform, and quinine, at the concentrations we used, recovery always took place sooner or later; but after aconitine, even at its lowest active concentration, no recovery was ever observed. The recovery began sooner after intoxication at low than at high temperatures, a result that we interpret as signifying that

Aconitine, *n*/10000. The action of the drug is suspended at low temperatures.

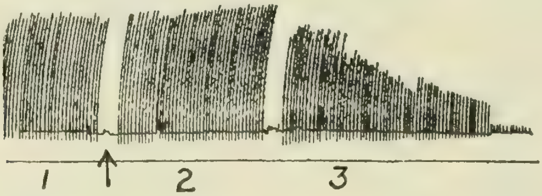
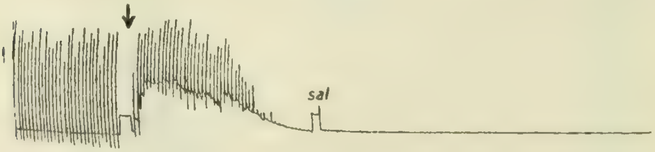


Fig. 16.—October 28, 1909. Three groups of contractions with the muscle: 1, in normal saline at 8°; 2, in aconitine hydrochloride, *n*/10000, at 8°; 3, in normal saline at 17°. During period 2, the aconitine diffused into the muscle; during the period 3, aconitine acted upon the muscle.

Fig. 17.—Effect of aconitine hydrochloride, *n*/1000, at 19°.



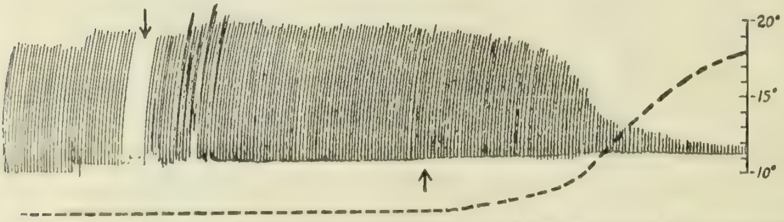


Fig. 18.—Effect of aconitine hydrochloride, *n*/1000. The action of the drug is suspended at a low temperature, 8°, as indicated by the broken line.

the toxic action had been more profound at high than at low temperature. But the recovery, having once commenced, proceeded more rapidly at high than at low temperature. We have not attempted to trace any quantitative relation between temperature and rate of recovery; we content ourselves with noting in this connection that after, *e.g.*, quinine $n/2000$, recovery began at once at 19°, but was delayed for five minutes at 25°, in another case, *i.e.* after alcohol $n/1$ at 20°, recovery began after 1 minute, and at 24° after 4 minutes. With a given toxic solution at a given temperature, the degree and completeness of recovery in saline were influenced by the previous duration of exposure to the action of the drug. Thus, *e.g.*, in alcohol $n/1$ the delay of recovery was 6 minutes after immersion for 12 minutes, and 10 minutes after immersion for 25 minutes.

An Examination of the Physical and Physiological Properties of Tetrachlorethane and Trichlorethylene.

By V. H. Veley, F.R.S.

(Received November 23, 1909,—Read January 20, 1910.)

(From the Physiological Laboratory of the University of London, South Kensington.)

Introductory.—Within the last few years chloroderivatives of the aliphatic hydrocarbons, other than the familiar chloroform, have been used for various purposes, occasionally with fatal results. It would, therefore, appear desirable to apply physiological tests, in the first instance, to such compounds to ascertain whether they might reasonably be scheduled as poisons, before they are entrusted to the hands of unskilled and ignorant persons. As two such substances, namely (1) one of the isomeric tetrachlorethanes, and (2) trichlorethylene, came into my hands through Dr. Willcox, they were compared with chloroform (taken as a standard) by the muscle method; further, as the physical data at least of the latter compound have been curiously overlooked, the opportunity was taken of determining certain of these by instruments, calibrated for previous investigations.

Historical.—Symmetrical tetrachlorethane, sometimes called acetylene perchloride, $CHCl_2.CHCl_2$, was first obtained by Berthelot and Jungfleisch* from acetylene and chlorine gases in presence of antimony chloride; the substance is described as a liquid, b.p. 147, soluble in alcohol, insoluble in water. These writers also showed that when this compound is heated with alcoholic potash, one molecular proportion of hydrochloric acid is removed with formation of trichlorethylene, $CHCl : CCl_2$, a liquid, b.p. 88, of chloroform-like odour.

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* 'Compt. Rend.,' 1869, vol. 69, p. 542.

Tetrachlorethane was more fully examined by Paterno and Pisati,[*] who prepared it from dichloraldehyde and phosphorus pentachloride; the following physical data are given, b.p. (corr.) $147 \pm 0·25$; $D^0 = 1·614$, $D^{24} = 1·578$, $D^{100} = 1·522$ compared with water at the same respective temperatures. They confirmed the observation of Berthelot and Jungfleisch as to the formation of trichlorethylene, b.p. 87 to 88.

Trichlorethylene was also obtained by Paterno and Oglialoro[†] as one of the products formed by heating chloral with phosphorus pentasulphide.

Certain physical data of tetrachlorethane are given by Kanonnikoff,[‡] namely $D^{26/4} = 1·5897$ and the refractive index $\mu_A = 1·49051$.

The preparation of tetrachlorethane on a sufficiently large scale by the chlorination of either ethylene chloride or acetylene (adopting certain precautions) in presence of aluminium chloride is fully described by Mouneyrat.[§] As the cost of manufacture, both of acetylene and chlorine gases, as also the price of aluminium chloride, have been considerably reduced, tetra-chlorethane can be prepared cheaply, as also trichlorethylene from it.

Tetrachlorethane.—The commercial product, fractionally distilled with a Young's still head with three bulbs, gave the following results:—

	Per cent.
Portion boiling below 138° (binary mixture of water and compound)	2·0
Ditto between 138° and 147° (probably trichlorethane)	9·5
Ditto at $147°·2 \pm 0·1$ (corr.)	77·0
Residue, rather discoloured	7·3
Loss	4·2
	100·0

The portion of b.p. 147°·2 was taken as pure, the boiling point being nearly identical with that found by Paterno and Pisati.

The densities of the liquid found were $D^{4/4} = 1·6208$ and $D^{17/17} = 1·6013_0$, the former value being rather higher than that of the above-named writers.

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[*] 'Gazz. Chim. Ital.,' 1871, vol. 1, p. 461.

[†] *Ibid.*, 1873, vol. 3, p. 538.

[‡] 'Journ. Russ. Phys. Chem. Soc.,' 1892, vol. 34, p. 602.

[§] 'Bull. Soc. Chim.,' 1898 [3], vol. 19, p. 452.

Trichlorethylene.—On distillation by the method described above, the following results were obtained. Bar. = 765:—

	Per cent.
Portion boiling below 87° (binary mixture of compound with water)	7·5
Ditto between 87° and 88°	80·5
Residue, very discoloured	7·5
Loss	4·5
	100·0

The portion boiling at 87° to 88° was allowed to stand some days over calcium chloride, and then redistilled; the main bulk of the liquid came over at $87·4 \pm 0·1$ (corr.). Another sample was previously shaken up with a concentrated solution of sodium carbonate, then dried over calcium chloride and redistilled; the main bulk of the liquid came over at $87·55 \pm 0·1$ (corr.). Both the samples were used as pure for the purpose of experiment.

I am indebted to Mr. Ellis for a determination of the chlorine contents; the value obtained was 99·97 of that required by theory, thus showing that the method adopted for purification had been successful.

As the statement has been made that trichlorethylene on distillation gives off hydrochloric acid gas, the receiver in the latter operation was connected with an absorption vessel, containing a definite volume of $n/10$ sodium hydroxide, but the amount of alkali neutralised by the gas evolved on distillation of 250 c.c. of trichlorethylene was quite insignificant. The vapour of trichlorethylene, kept in a stoppered bottle and exposed to ordinary daylight, gave an acid reaction, but the same applies in a less degree to chloroform.

At present, no experiments have been conducted with a view of determining the relative decomposition and consequent acidity of trichlorethylene and chloroform respectively of presumably the same degree of purity and under identical conditions of illumination.

The suggestion is, however, raised that the superincumbent vapour, and not the liquid, in a partially filled bottle is initially decomposed.

Determination of the density at different temperatures gave the following results:—

$$D^{4/4} = 1·4904_5,\ D^{17·5/17·5} = 1·4702_0,\ D^{25·5/25·5} = 1·4598_7;$$

from these determinations the relative volumes in terms of that at 4° are:—

$$V_4 = 1, \quad V_{17·5} = 1·0128, \quad V_{25·5} = 1·0209.$$

Refractive Indices of above Compounds.

I am indebted to Mr. J. J. Manley, M.A., of the Laboratory, Magdalen College, Oxford, for these determinations, made with the Becker-Meyerstein spectrometer and quartz prism described in previous communications.*

As the instrument and prism had been out of use for some time, it was thought desirable to remeasure the angle of the prism, and to determine the refractive index μ_D of "conductivity" water. The following values show that the apparatus had remained in perfect order:—

Angle of Prism.

Present values.	Past values.
60° 6′ 8″ to 60° 6′ 24″.	60° 6′ 0″ to 60° 6′ 24″.

Refractive Index of "Conductivity" Water.

$\mu_D = 1{\cdot}333400 \pm 0{\cdot}0_54$.	$\mu_D = 1{\cdot}333393$.
$K_{cm.}{}^{-1}\,ohm^{-1} = 1{\cdot}3\,.\,10^{-6}$.	$K_{cm.}{}^{-1}\,ohm^{-1} = 1{\cdot}2\,.\,10^{-6}$.

Tetrachlorethane.—Five independent determinations of μ_D, made at temperatures 15°·2 to 17°·3, and corrected to a standard temperature 17°, gave the following results:—

(1)	1·495602	(3)	1·495595
(2)	582	(4)	550
(5)	1·495607		

The above lead to a final value $1{\cdot}495587 \pm 0{\cdot}0_56$ calculated by Bessel's function.

The determinations give for Gladstone's factor $\mu - 1/d = 0{\cdot}3095$, and for Lorenz' factor $\mu^2 - 1/(\mu^2 + 2)\,d = 0{\cdot}1824$; and if multiplied by the molecular weight 53·0 (50·6 Kanonnikoff), and 30·7 respectively.

Trichlorethylene.—Six independent determinations of μ_D at temperatures varying from 15·2 to 17·3 (Nos. 1 to 4 with one portion, 5 to 6 with another); these, corrected to standard temperature, 17°, gave the following results:—

(1)	1·479144	(4)	1·479125
(2)	38	(5)	31
(3)	50	(6)	56

The above led to a final value $1{\cdot}479141 \pm 0{\cdot}0_53$, calculated as before.

The determinations give 0·326 for Gladstone's factor, and 0·193 for Lorenz' factor, which, when multiplied by the molecular weight, give 42·7 and 25·8 respectively.

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* 'Roy. Soc. Proc.,' 1901, vol. 69, p. 105; *ibid.*, 1905, A, vol. 76, p. 472.

Preparation of Solutions for Physiological Experiments with Frog's Sartorius Muscle by Waller's Method.[*]

The standard taken for comparison was $n/100$ chloroform, and all data are given in terms thereof; the chloroform sample used had been prepared from chloral hydrate. Solutions of chloroform, tetrachlorethane, and trichlorethylene were made up in physiological saline solution (0·6 per cent.) to $m/100$ concentration by weighing out to within 1 part in 800 the quantities of the several liquids required, which would be as under:—

1·195 gramme chloroform in 1 litre = 0·119 per cent. (neglecting density variation of water).
1·315 „ trichlorethylene „ = 0·131 „ „
1·678 „ tetrachlorethane „ = 0·168 „ „

Half these quantities were actually taken and dissolved in $\frac{1}{2}$ litre saline solution. The chloroform and trichlorethylene dissolved fairly readily in this proportion, but the tetrachlorethane required a day or more for completion.

It was found necessary to prepare the chloroform solution afresh after two or three days, as otherwise irregular results were obtained; but whether this was the result of loss of chloroform by evaporation, or some chemical change between the chloroform and water in presence of daylight, or from both causes, was not further investigated.

In the preliminary experiments with the commercial products the substances were weighed out as pure for lack of better data; the errors would probably amount to 8 to 10 per cent.

The experiments were conducted in the usual manner; simultaneous records with a pair of muscles were obtained: firstly, of the normal response to induction shocks when the muscles were immersed in tap-water saline solution (0·6 per cent.); secondly, the time required for abolition of response after the solutions to be examined had been simultaneously substituted for the saline solution, and, thirdly, the time and degree of recovery, if any, after the saline solution was in its turn resubstituted for the solutions under examination.

Preliminary experiments with commercial samples of trichlorethylene and tetrachlorethane showed that the former was rather more toxic, but more regular in its action, than chloroform, and that the latter was about four times more toxic than chloroform; in both cases the recovery was more regular. The details with the purified materials are given in the sequel:—

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[*] 'Roy. Soc. Proc.,' June, 1909.

(1) Tetrachlorethane.

Solutions.	Times of abolition.	Recovery.
First series of experiments.		
	mins.	
Tetrachlorethane, *n*/200	10	Slow, but fairly complete.
Chloroform, *n*/100	17	Fairly complete.
Second series.		
Tetrachlorethane, *n*/400	16·5	Good.
Chloroform, *n*/100	16	Rather irregular.
Third series at lower temperature.*		
Tetrachlorethane, *n*/400	25	Fair.
Chloroform, *n*/100	25	Rather irregular.

* The effect of temperature had not been investigated when these experiments were performed; it is, of course, immaterial for comparative experiments with the same pair of muscles at the same temperature.

The records obtained in the second series are given in figs. 1 and 2.

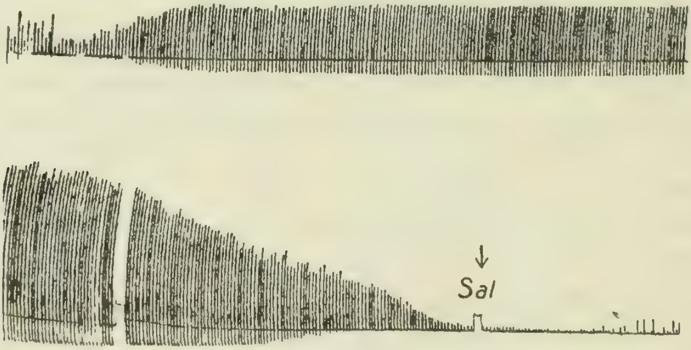

FIG. 1.

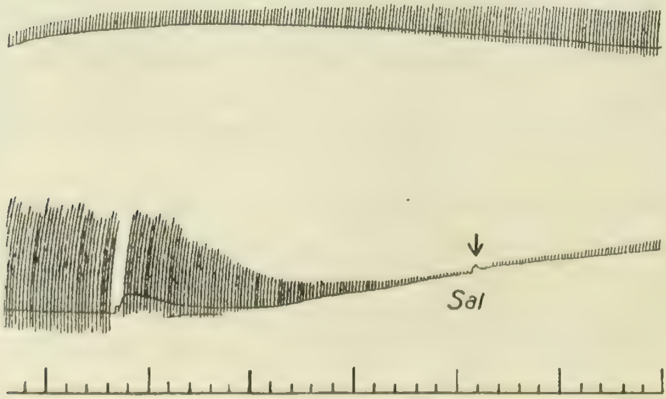

FIG. 2.

Hence, therefore, tetrachlorethane is four times more toxic than chloroform, molecule for molecule, but recovery from anæsthesia or paralysis is more regular in the case of the former than in that of the latter.

Trichlorethylene.

Solutions.	Times for abolition.	Recovery.
First Series.		
	mins.	
Trichlorethylene, *n*/100	9·5	Delayed for some time, then fairly good.
Chloroform, *n*/100.....................	17·0	Fairly good.
Hence ratio chloroform to trichlorethylene = 1 : 1·8.		
Second Series.		
Trichlorethylene, *n*/200	24·5	Good.
Chloroform, *n*/100.....................	17·0	Fair.
Hence ratio chloroform to trichlorethylene = 24·5/2 : 17 = 1 : 1·5.		
Third Series.		
Trichlorethylene, *n*/150	25·0	Good.
Chloroform, *n*/100.....................	25·0	Fair.
Fourth Series, repetition of Third, at higher temperatures.		
Trichlorethylene, *n*/150	17·0	Good.
Chloroform, *n*/100.....................	17·0	„

The records obtained in the third series are illustrated in figs. 3 and 4.

Hence, trichlorethylene is 1·5 times more toxic than chloroform, molecule for molecule, or 1·36 times, weight for weight. It will be observed on

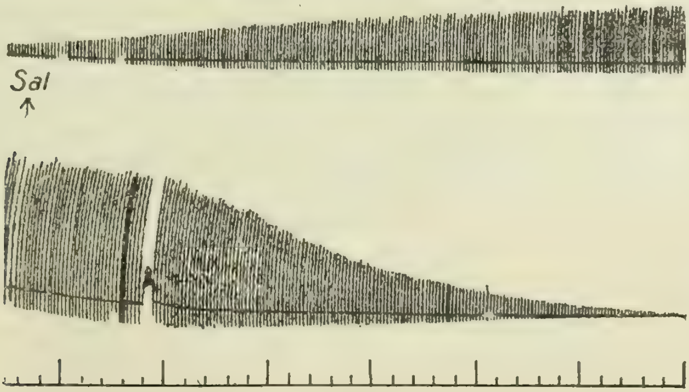

FIG. 3.

inspection of figs. 3 and 4 that not only the course of abolition, but also of recovery, are much more regular in the case of trichlorethylene than in the case of chloroform. Accordingly exact measurements were made of the amount of response expressed in millimetres, the outlines for the purpose being copied out on squared tissue paper.

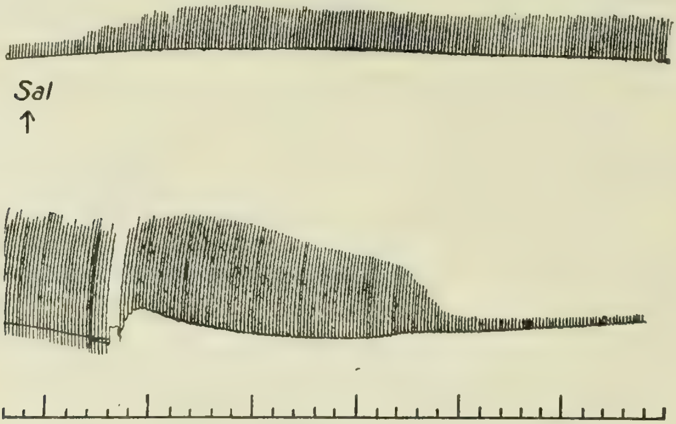

Fig. 4.

Taking, then, these heights in the course of abolition as a measure of the action, whether chemical or otherwise, of the trichlorethylene and chloroform respectively on the isolated muscle, the results are as set out below. In the first column are given the times in minutes from start; in the second, fourth, and sixth columns the observed heights in millimetres; in the third the values calculated from equation $\Delta x/\Delta t$ = constant = 1; and in the fifth similar values from equation $\Delta x/\Delta t$ = constant = 1·5. The rate of chemical change would, of course, be the reciprocals of these numbers.

	Trichlorethylene.				Chloroform.	
I.	II.	III.	IV.	V.	VI.	VII.
0	18	17	20	20	15	15
2	16	15	17	17	14	16
4	13	13	13	14	13·5	15·5
6	11	11	11	11	12	13·5
8	9	9	7	8	10	10
10	7	7	5	5	5·5	5
12	6	5	2	3	3	3
14	4	3	2	1	1	2
16	3	1	—	—	1	1
18	2	—	—	—	0·5	0·5
20	2	—	—	—	0·5	0·5

It will be observed from the above numbers that the action of trichlorethylene on the muscle can be represented practically, except at the end, by a straight line,* the geometrical figure being approximately a right-angled triangle, whereas that of chloroform is initially an area bounded by a convex and a concave surface, and finally becomes triangular.

Though cases in which the rate of chemical change is a linear function of time are rare, yet they have been observed, especially with solutions of very great dilution.† Such a result would not, therefore, exclude the action of trichlorethylene on muscle from the category of chemical change.

As regards the recoveries, that from the chloroform was, at first, more pronounced, but generally a diminution set in, and in some cases a contracture more or less marked, which put an end to the record. On the other hand, that from the trichlorethylene started slowly and proceeded regularly. The measurements of one such recovery are given; at the point called "interval" it was necessary to reset the travelling plate and marker for a higher line; the time would be about two to three minutes.

Time.	Recovery in millimetres (observed).	Time.	Recovery in millimetres (observed).	Time.	Recovery in millimetres (observed.)
0	0	0	6	12	9·5
2	3	2	7*	14	9·5
4	4	4	7·5	16	10
6	5	6	8·0	18	10·5
Interval		8	8·5	20	11
		10	9	22	11·5

From these figures it is clear that after the first interval the recovery increases uniformly at the rate of 1 mm. per two minutes at the earlier stage, and of 0·5 mm. per two minutes at the latter stage, the difference between these two rates being probably an effect of fatigue. But, however this may be, the recovery from trichlorethylene is more regular than that from any other drug examined by this method.

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* From an inspection of numerous records it appears probable that an arc of a curve has become almost coincident with its chord; the result is quite analogous with certain cases of chemical change.

† Veley, 'Chem. Soc. Trans.,' 1909, vol. 95, p. 1188.

The Photochemical Formation of Formaldehyde in Green Plants.

By S. B. Schryver, D.Sc., Ph.D., Lecturer on Physiological Chemistry in University College, London.

(Communicated by Prof. E. H. Starling, F.R.S. Received November 13, 1909,—Read January 27, 1910.)

Since von Baeyer propounded his theory of the formation of formic aldehyde as an intermediate product in the synthesis of sugars from carbon dioxide and water by green plants, many attempts have been made to discover this substance, and to substantiate Baeyer's theory. The most successful of these are due to Polacci,* and to Usher and Priestley.† More recently R. J. Harvey Gibson, in conjunction with A. W. Titherley, has adduced further evidence tending to show that formaldehyde is actually formed in green plants, and the former has propounded a photo-electric theory to account for the photochemical action.‡

Nevertheless, the evidence hitherto brought forward has been generally considered, from an experimental point of view, somewhat inconclusive, and most of the above-quoted investigations have been subjected to numerous criticisms.

It is hardly necessary to examine these criticisms in detail here, nor to discuss the various theories that have been advanced to explain the mechanism of the reaction,§ as it is thought that the experiments recorded in this paper will support an alternative hypothesis, by means of which some of the objections to the original conception of Baeyer may be removed.

Method.

During the course of some investigations on formaldehyde in foodstuffs‖ a method was devised, by means of which both the free and combined aldehyde could be readily detected and quantitatively estimated, even when the amount present did not exceed one part in a million. The possession of this method afforded the opportunity for the re-investigation of the vexed question of the photochemical synthesis of the aldehyde by green plants.

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* 'Arch. Ital. Biolog.,' vol. 35, p. 151, and vol. 37, p. 446.

† 'Roy. Soc. Proc.,' vol. 77, p. 369, and vol. 78, p. 318.

‡ 'Annals of Botany,' 1908, vol. 32, p. 117.

§ See Polacci, 'Accad. Lincei Roma,' June 6, 1908, and Ewart, 'Roy. Soc. Proc.,' 1908, vol. 80, p. 30.

‖ Report No. 9, Inspector of Foods Department of the Local Government Board.

The reaction employed for the purpose of estimation was, in its first form, originally described by Rimini, who showed that when formaldehyde solutions were treated with phenylhydrazine hydrochloride, and a drop of ferric chloride and concentrated sulphuric acid were subsequently added, a brilliant fuchsine-like colour developed. The reaction in this form was found to be uncertain; in the presence of too small a quantity of ferric chloride, the full colour due to the reaction was not developed, whereas in the presence of an excess of the reagent, the colour was rapidly destroyed. The use of concentrated sulphuric acid was also inconvenient when the reaction was employed quantitatively.

The reaction is due to the formation of a condensation product of formaldehyde and phenylhydrazine, which, on oxidation, yields a weak base; the latter, in the presence of excess strong acids, yields salts, which readily undergo hydrolytic dissociation on dilution. By substituting for ferric chloride an oxidising agent, which, when added in excess, does not destroy the colour, and by employing concentrated hydrochloric acid instead of sulphuric acid for the formation of the coloured salt, the Rimini reaction can be adapted to quantitative estimation of formaldehyde. In this form one part of formaldehyde in one million can be readily detected, whereas Rimini claimed that his original test was only sufficiently sensitive to detect one part in 50,000.

The test, with the above modifications, is carried out in the following way. To 10 c.c. of the formaldehyde-containing liquid are added 2 c.c. of a 1-per-cent. solution (freshly made up and filtered) of phenylhydrazine hydrochloride; 1 c.c. of a fresh 5-per-cent. solution of potassium ferricyanide is then added. On addition of 5 c.c. concentrated hydrochloric acid, a brilliant colour is developed in presence of formaldehyde; by comparing the depth of colour with that of standard solutions (of concentrations between 1 in 1,000,000 and 1 in 100,000), the quantity of formaldehyde present can be determined.

By the test in the above form, free formaldehyde can be detected and, when sufficiently dilute, quantitatively estimated. By a slight modification formaldehyde in a polymerised form, as in the so-called oxymethylene, or in the form of stable combination, as in hexamethylenetetramine (urotropine), can also be detected and estimated. If to a weak solution (10 c.c.) of hexamethylenetetramine be added phenylhydrazine hydro- and ferri-cyanide solutions, and then hydrochloric acid in the quantities given above, only a faint coloration (if any) will develop. If, however, the mixture be warmed for a short time, after addition of the phenylhydrazine hydrochloride solution, or be allowed to stand, and the ferricyanide solution and acid be then added, the full colour due to the combined formaldehyde will develop; the hexamethylenetetramine in the presence of water undergoes slight hydrolysis into ammonia and formaldehyde, and as the latter can be removed from the sphere of action in the presence of phenylhydrazine, the reaction in the presence of this reagent can proceed to completion, and the total combined formaldehyde can be thereby estimated. This reaction will be discussed in greater detail below, when considering the mechanism of the formaldehyde formation of chlorophyll.

In the presence of other pigments, the method of carrying out the reaction can be conveniently modified, especially when the quantities of formaldehyde present are only small. The reaction mixture, after addition of the reagents (phenylhydrazine and ferricyanide reagents and hydrochloric acid), is diluted with water in a small separating funnel, and ether is added. After shaking up, the hydrochloride of the chromatogenic base is dissociated, and the free base passes into the ethereal solution; the latter is separated off from the underlying aqueous layer. On addition of concentrated hydrochloric acid to the ethereal solution, the base readily passes into the acid, in the form of the coloured hydrochloride. By using only a small quantity of acid, the sensitiveness of the test is largely increased, as the colour can be finally contained in only 1 or 2 c.c. of solution, instead of 18 c.c. as required in the original test. The test in this form has been applied in investigating the synthesis of formaldehyde by chlorophyll.

The test appears to be characteristic for formaldehyde. Commercial solutions of acetaldehyde yield a very faint pink colour, when in relatively strong solutions (1 to 2 in 1000), but this is due to contaminations with small quantity of formaldehyde, derived from methyl alcohol. Furfuraldehyde, which can certainly be obtained from plants, gives a characteristic reaction when present in sufficient concentration. On addition of phenylhydrazine and ferricyanide reagents to furfuraldehyde solution (1 in 1000) an apricot yellow colour is formed, which turns a dirty-green colour on addition of concentrated hydrochloric acid; this, however, is only transient, and rapidly disappears. If, shortly after addition of the acid, and before the disappearance of the green colour, the reaction mixture be diluted and extracted with ether, and the ethereal solution be then extracted by acid, the latter takes up a green pigment. This reaction, however, is only obtained, if the dilution and extraction with ether be made very shortly after addition of the acid, and in this respect (as also in colour) it differs from the formaldehyde pigment, which is fairly stable. Furfuraldehyde, furthermore, gives no reaction with the reagents employed in the formaldehyde test when the dilution reaches 1 in 100,000. The reaction, to be described below, yielded by chlorophyll, can only be due, therefore, to formaldehyde.

Presence of Formaldehyde in Chlorophyll.

Grass was always employed as a source of chlorophyll in the following tests. It was first washed with warm water, and no formaldehyde was ever detected in the aqueous washings. It was then pressed by hand, to free it, as completely as possible, from the wash water, and extracted with methylated spirit. After standing for some time (generally over-night) the alcohol was filtered off on a Buchner funnel. Several times the alcoholic extract was tested directly for formaldehyde. It was evaporated to dryness, and the residue warmed with 10 c.c. of water and 2 c.c. of the phenylhydrazine reagent. The ferricyanide reagent and hydrochloric acid were then added. In no case did a colour develop. If, however, the alcohol were evaporated off *in vacuo*, and the residue taken up by ether, and the ether were then distilled off, a residue was obtained, which in certain cases gave a positive result when examined for formaldehyde. The reason for this behaviour was found to be due to the fact that the alcoholic extract contained substances, probably sugars, which interfered with the formaldehyde reaction, for after evaporating off the alcohol *in vacuo* at about 40° and extracting with ether, a residue insoluble in the latter solvent was obtained, which readily reduced Fehling's solution. For this reason, the chlorophyll must always be purified by solution in ether before carrying out the test.

In most of the samples of chlorophyll examined, formaldehyde was found. These were collected, however, during the summer season, after a long spell of daylight. A sample collected early on a foggy morning, after little sunshine, contained only a trace of formaldehyde.

In carrying out the test for formaldehyde with chlorophyll, the latter had been extracted with alcohol; this extract had been evaporated to dryness, and the residue thus obtained extracted with ether. The ethereal solution was itself evaporated to dryness. The formaldehyde reaction was then only obtained after allowing a film of the chlorophyll* obtained by evaporating the ethereal solution on a glass plate, either to stand for some time with a cold mixture of 10 c.c. water and 2 c.c. of a 1-per-cent. phenylhydrazine hydrochloride solution, or by warming the same mixture for a few minutes to 100° C. On addition of the ferricyanide solution and acid, the characteristic colour then developed.

The fact that formaldehyde could be detected in chlorophyll after repeated evaporation of the solutions, and that warming or standing with the phenyl-

---

* The crude product thus obtained is hereafter called chlorophyll, and is probably a mixture of several substances.

hydrazine reagent was necessary, indicate that the aldehyde is contained in the chlorophyll in the state of somewhat stable combination.

The Photochemical Formation of Formaldehyde by Chlorophyll.

In the earlier researches on this subject by Usher and Priestley, the formation of aldehyde was demonstrated by allowing chlorophyll films extended on gelatin plates, or on the surface of water, to remain exposed to light in the presence of carbon dioxide. It is conceivable that, in the experiments carried out in the first-named manner, some of the aldehyde may have been derived from the gelatin.* In the experiments described below, the formation of formaldehyde can be demonstrated when films of chlorophyll alone are employed.

It has been already stated that a sample of chlorophyll from grass collected on a foggy morning contained only very small traces of the aldehyde. Furthermore, formaldehyde gradually disappeared on keeping from ethereal solutions of chlorophyll, which were originally rich in the aldehyde. The experiments were carried out with such formaldehyde-free chlorophyll solutions.

Each test was carried out with 1 c.c. of an ethereal solution of chlorophyll, which was allowed to evaporate at room temperature on a strip of glass (about 140 by 20 mm.). Some films thus prepared were kept in the dark, others were exposed to moist carbon dioxide in sunlight, others to sunlight over lime or soda lime, and others, again, were exposed to moist carbon dioxide in the dark. For testing for formaldehyde two films were usually employed, and in each case either warmed for a few minutes or allowed to stand for some hours with a mixture of 10 c.c. water and 2 c.c. of the phenylhydrazine reagent, and the test was then completed in the usual manner. The results are tabulated below.

Conditions of experiment.	Result.
Kept in the dark either in presence or absence of moist CO_2	No formation of HCHO
Kept in sunlight over lime or soda lime	Formation of very minute quantities of HCHO
Kept in sunlight over moist CO_2..................	Formaldehyde reaction distinct

It will be seen that formaldehyde is formed when the chlorophyll film is exposed to sunlight either in presence or absence of carbon dioxide. In the latter case, the quantity of aldehyde formed is so minute that its presence could only be demonstrated with certainty after dilution of the reaction mixture with water, extraction with ether, etc., in the method given above (see p. 228) for the modified formaldehyde test. In this case, carbon dioxide or formaldehyde was probably formed by the photochemical decomposition of other substances in the crude chlorophyll, for Neuberg has recently shown that a large number of organic substances readily undergo decomposition on exposure to light in the presence of optical sensitisers, such as uranium salts.* When carbon dioxide was present, the formaldehyde reaction was visible when the ordinary test was applied, without having to dilute with water and extract the chromatogenic base with ether. In no case, however, was the formation of formaldehyde demonstrable when the films were kept in the dark. These experiments were carried out several times with different chlorophyll preparations, and yielded always the same results. Only very small quantities of chlorophyll need be employed in each experiment. In the quantities employed for each of the experiments described above, no formaldehyde could be detected in the original film by any of the given methods of testing.

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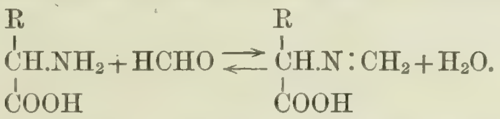
* Compare Meisling, 'Revue Générale des Sciences,' May 15, 1909

Mechanism of the Reaction.

It has been generally objected to the conception of the formation of formaldehyde as an intermediate product of sugar synthesis in plants, that this substance is highly toxic. This objection has, however, lost some of its weight as a result of the experiments of Tréboux,† who states that certain plants, when placed in very dilute solutions of formaldehyde, can synthesise therefrom starch.

The fact that formaldehyde after synthesis by chlorophyll exists in the state of fairly stable combination with some product or group, explains how the quantity of the aldehyde present can be automatically regulated. The action of formaldehyde on various organic substances, especially those containing the amino-group, has been studied by Schiff.‡

He has shown that in many cases the reaction between an amino-group and the aldehyde is a reversible one, as in the case of the amino-acids—



$$\begin{array}{l}\mathrm{R}\\ |\\ \mathrm{CH.NH_2+HCHO}\\ |\\ \mathrm{COOH}\end{array} \rightleftharpoons \begin{array}{l}\mathrm{R}\\ |\\ \mathrm{CH.N:CH_2+H_2O.}\\ |\\ \mathrm{COOH}\end{array}$$

In some cases this reaction proceeds to completion only in the presence of a large excess of the aldehyde.§ In other cases, as in that of hexamethylenetetramine, the hydrolysis of the methyleneamino-derivative, which is a somewhat stable compound, is only very small (the reaction represented by the arrow pointing to the left in the above equation is only very incomplete). In such cases, the formaldehyde reaction can only proceed to completion after warming or allowing the mixture to stand for some time in the presence of the phenylhydrazine reagent. The hydrolysis proceeds to completion, that is to say, when the formaldehyde that is set free is removed from the sphere of action. A similar stable compound appears to be formed with the chlorophyll* and formaldehyde. The reaction may be schematically represented by the following equation :—

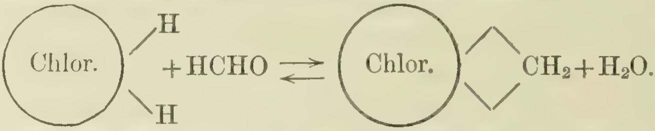
---

* 'Biochem. Zeitsch.,' 1908, vol. 13, p. 305.

† 'Flora,' 1903, p. 73.

‡ 'Liebig's Annalen,' 1899, vol. 310, p. 25; 1901, vol. 319, pp. 58 and 287; and 1902, vol. 325, p. 348.

§ Compare Sörensen, 'Biochem. Zeitsch.,' 1908, vol. 7, p. 45.



As the condensation product is somewhat stable, equilibrium will be maintained when only a very small amount of free aldehyde is present. As this is removed by synthesis into sugars, etc., more of the chlorophyll-aldehyde condensation product will decompose. It can furthermore be reconstituted in the presence of carbon dioxide and sunlight. The fact, therefore, that such a stable compound is formed, which undergoes only limited hydrolysis, explains the mechanism by means of which the quantity of formaldehyde in the plant available for sugar synthesis can be regulated. In the presence of sunlight and carbon dioxide, there is probably a continuous synthesis of formaldehyde, and a continuous condensation of the latter to sugars, without at any time an accumulation of the aldehyde in such quantity as to be toxic to the plant. It is possible that the synthetic formation of sugar is due, as Usher and Priestley have suggested, to the living protoplasm, as this hypothesis explains certain phenomena to which they have called attention in their paper.

Summary.

1. A reaction is described by means of which minute quantities of formaldehyde, both combined and free, can be detected and estimated.

2. Formaldehyde can be detected in chlorophyll, and exists therein in a state of combination.

3. By means of the reaction described, the photochemical synthesis of formaldehyde by chlorophyll can be demonstrated.

4. From the fact that formaldehyde exists in a state of combination in chlorophyll, it is possible to explain how the supply of aldehyde necessary for sugar synthesis in the plant is regulated.

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* See note, p. 229.

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ON THE REFRACTION AND DISPERSION OF SULPHUR DIOXIDE AND HYDROGEN SULPHIDE, AND THEIR RELATION TO THOSE OF THEIR CONSTITUENTS. By Clive Cuthbertson and Maude Cuthbertson 171

ON THE NATURE OF THE HYDROGEN FLOCCULI AND THEIR STRUCTURE AT DIFFERENT LEVELS IN THE SOLAR ATMOSPHERE. By George E. Hale, For. Mem. R.S., and Ferdinand Ellerman. (Plate 1) 177

ON THE DISTRIBUTION OF THE RONTGEN RAYS FROM A FOCUS BULB. By G. W. C. Kaye, B.A. (Cantab.), D.Sc. (Lond.), A.R.C.Sc. 189

ON THE NATURE OF THE IONISATION OF A MOLECULE BY AN α-PARTICLE. By R. D. Kleeman, D.Sc., B.A. 195

THE CRYSTALLINE STRUCTURE OF IRON AT HIGH TEMPERATURES. By Walter Rosenhain, B.A., D.Sc., and J. C. W. Humfrey, B.A., M.Sc. (Plate 2) 200

THE SPECTRUM OF MAGNESIUM HYDRIDE. By Prof. A. Fowler, A.R.C.S., F.R.A.S. (Abstract) 210

THE RELATION OF THALLIUM TO THE ALKALI METALS: A STUDY OF THALLIUM-ZINC SULPHATE AND SELENATE. By A. E. H. Tutton, M.A., D.Sc., F.R.S. .. 211

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CONTENTS.

Page

Further Observations on the Pathology of Gastric Ulcer. (Progress Report.) By CHARLES BOLTON, M.D., D.Sc., F.R.C.P., Director of the Research Laboratories, University College Hospital Medical School, Assistant Physician to University College Hospital. (Plates 8 and 9) . . . 233

On the Action of Antimony Compounds in Trypanosomiasis in Rats: being a Report to a Sub-Committee of the Tropical Diseases Committee of the Royal Society. By JOHN D. THOMSON, M.B., C.M., and ARTHUR R. CUSHNY, M.D., F.R.S. 249

Amakebe: A Disease of Calves in Uganda. By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. Sleeping Sickness Commission of the Royal Society, 1908—09. (Plate 10) 256

The Transmission of Trypanosoma lewisi by the Rat-flea (Ceratophyllus fasciatus). (Preliminary Communication.) By E. A. MINCHIN, M.A., and J. D. THOMSON, M.A., M.B., C.M. 273

On the Relative Sizes of the Organs of Rats and Mice bearing Malignant New Growths. By Dr. F. MEDIGRECEANU (Bucharest) . . . 286

The Contrast in the Reactions to the Implantation of Cancer after the Inoculation of Living and Mechanically Disintegrated Cells. By M. HAALAND, M.D., Imperial Cancer Research Fund 293

Further Evidence on the Homogeneity of the Resistance to the Implantation of Malignant New Growths. By E. F. BASHFORD, M.D., and B. R. G. RUSSELL, M.D., Imperial Cancer Research Fund 298

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(From the Research Laboratories, University College Hospital Medical School.)

[Plates 8 and 9.]

The gastric ulcers in these experiments were produced by the injection of gastrotoxic serum. In a former communication (1) it was demonstrated that the serum, formed by immunising the rabbit with the gastric cells of the guinea-pig or with those of another rabbit, on injection into the guinea-pig's peritoneum produced general symptoms of intoxication and patches of necrosis in the mucous membrane of the stomach. In two later communications (2) and (3) the reactions of gastrotoxin *in vitro* and its precise mode of action *in corpore* were demonstrated.

In a fourth paper (4) the healing of gastrotoxic ulcers was dealt with, the method adopted in this series of experiments being that of injection of the serum directly into the stomach wall, so that it attacked the gastric cells directly and not through the blood stream, and in this way an ulcer was produced without general symptoms of intoxication. Briefly stated, it was found that such ulcers invariably healed within three or four weeks, and that, so long as the stomach emptied itself in the normal time, any moderate alteration in the acidity of the gastric contents did not delay the healing of the ulcers.

Chronic gastric ulcer in the human subject is, however, a common malady and, since ulcers formed by the process of self-digestion are initially acute, it was considered that there must be present some unknown condition or conditions which prevents the healing of such chronic ulcers.

The present communication deals especially with the effects of motor insufficiency of the stomach upon the healing of gastric ulcer.

In order to study the effects of motor insufficiency, it is necessary to use an animal which eats definite meals and has definite resting periods between. The cat was chosen as a suitable animal.

I. *Production of a Gastrotoxin Active against the Cat.*

1. *Method.*—The gastric cells of the cat were periodically injected into another animal, the blood of the latter gradually becoming poisonous for the cat's gastric mucous membrane. In this way I have endeavoured to immunise the rabbit, fowl, and goat. The rabbit and fowl proved very difficult animals to work with, as they very readily succumbed to the injections and could only resist very small doses.

Immunisation of the Goat.—I found that the goat was quite a suitable animal, and that it produced a very powerful gastrotoxin for the cat. Subcutaneous injections of cat's stomach cells were given at intervals of 7 to 10 days. The development of the immune substances in the blood of the goat is the same as I have before described in connection with the guinea-pig-rabbit gastrotoxin.

At first I injected the cells themselves, but found that abcesses were liable to result. I then tried an emulsion of cells in a solution of salicylic acid with a similar result. I now use a fresh dilute saline extract and give 50 c.c. for a dose. This very rarely produces suppuration if due care be exercised. The injections are given as I have described before, and the goat is bled from the ear if a small quantity of blood be required, but from the jugular vein with a cannula if a large quantity be needed.

I have used the blood serum so obtained as a means of producing an ulcer of a definite size and in a definite position in the cat's stomach.

2. *Effects of Local Injection of the Serum.*—The method of local injection into the cat's stomach wall is the same as I described in connection with the guinea-pig. The animal is anæsthetised with ether and the abdomen opened under antiseptic precautions. The stomach is drawn out and a hypodermic needle inserted between the mucous and peritoneal layers of the stomach. The serum is slowly injected with a 10-c.c. glass syringe. A local œdema is thus produced which forms a button-like thickening in the wall and projects into the lumen of the stomach as a rounded elevation.

If cut across, the fluid appears to be infiltrating the muscular coat. I have tried injections between the muscular and peritoneal coats and also between the muscular and mucous coats, but the result always appears to be the same, and I have not found it possible to destroy definite layers of the stomach wall in this way. It might be possible to do so in the case of a larger animal.

The amounts I have injected are from 5 to 10 c.c. I now employ 6 or 7 c.c. when I wish to produce an ulcer. The serum is rapidly absorbed, and apparently soaks into the overlying mucous membrane, which is then digested by the gastric juice. In all the following experiments the serum was injected about the middle of the anterior wall at the cardiac end of the stomach. The animals, as a rule, do not eat well for a day or two, and may vomit once or twice and lose a little weight, but they soon recover and do not appear to suffer from any symptoms.

Formation.—The part of the mucous membrane affected sloughs, and by the third to the fifth day a clean ulcer results, which may involve the mucous membrane only, or may extend through the submucous and muscular coats. The depth depends upon the strength of the serum and upon the condition of the contents of the stomach. Perforation of the ulcer and resulting peritonitis occasionally occurs.

The acute ulcer so formed has cleanly defined margins and base, and is rounded or sometimes more irregular in shape. The whole of the area of muscular coat exposed may have disappeared or only a portion of it. In short, the ulcer presents the typical punched-out appearance of the acute gastric ulcer of man.

3. *Mode of Action of the Serum.*—As I have said, the action is a toxic one. The serum causes changes in the cells of the overlying mucous membrane, which is then digested by the gastric juice. That the action is not a mechanical one I have proved by injecting neutral fluids, as I have described previously in the case of the guinea-pig.

Ten cubic centimetres of cat's serum may be injected into the stomach wall, and whether the stomach is resting or digesting the serum is completely absorbed, and no ulcer results. Further, the effect produced on the stomach and the extent of the ulceration depend upon the stage of immunisation to which the goat has reached. So that there is a definite poison in the serum which directly affects the gastric cells in the same way as the guinea-pig-rabbit serum affects the cells of the guinea-pig.

4. *Dependence of the Extent of Ulceration upon whether the Stomach is Resting or Digesting.*—The necrotic lesions in the gastric mucous membrane being dependent upon the action of the gastric juice, it would appear that when the gastric glands were resting and the stomach empty the lesions should fail to appear or at all events be less marked than when the organ contained food, unless the gastrotoxin has the power of exciting secretion, a point which I have not yet investigated. I did a series of experiments on guinea-pigs, half the animals being starved and half fed, to settle this question, and found that I was unable to do so, because it is practically impossible in my experience to obtain a guinea-pig with its stomach absolutely empty. These animals will not live very long without food, and 24 hours after feeding the stomach contains a fair amount of highly acid fluid with food remnants.

In the case of the cat I have been able to prove definitely that when the stomach is empty the ulcer may not appear at all, and if it does that it only extends down to the submucous tissue, as a rule. Of nine experiments, ulceration failed to appear in three, and in the remaining six was only superficial. Even though the stomach be empty, there is often a little acid fluid in it, and this accounts for lesions occurring in the empty stomach.

I may point out also that the secretion of gastric juice not only depends upon the presence of food in the stomach, but is also a reflex nervous phenomenon. The cat has a very keen scent, and it is difficult to exclude the smell of food, which excites a flow of gastric juice. Moreover, I do not know how long the effect of the poison upon the cells lasts, and if food be given before this effect passes off, ulceration will of course occur.

This is a matter of no small importance, because when the process of ulceration is actively going on, one point in the treatment of such a case is obviously to stop the flow of gastric juice, not to encourage it by giving a diet such as recommended by Lenhartz (5); I have shown before that whenever the stomach contains food, even though the food be strongly alkaline, there is always a layer having a strongly acid reaction in contact with the wall of the stomach.

In all the following experiments, 15 grammes of cut-up meat were given to each cat two hours before the operation for the production of ulcer.

II. *The Healing of Acute Gastric Ulcer in the Cat.*

The healing stages have been studied in 21 cats on a normal diet. After the 3rd to the 5th day healing commences and is completed in many cases before, and nearly always by, the 21st day.

The edges of the ulcer are drawn together by contraction of the muscular coat of the stomach, and fixed in this position by the formation of fibrous tissue. Puckering of the surrounding mucous membrane is thus produced, and a stellate scar results. The amount of the puckering depends upon the previous degree of contraction of the muscular coat, and the shrinkage of the fibrous base of the ulcer (Plate 8, fig. 1). Sometimes the edges of the mucous membrane are curved inwards all round, probably by retraction of the muscularis mucosæ. The pressure of this edge interferes with the normal regeneration of the mucous membrane. The peritoneum is thickened to different degrees, and may be adherent to the omentum, liver, or diaphragm.

The regeneration of the gastric mucous membrane was fully worked out microscopically by Griffini and Vassale (6) 20 years ago. They cut off portions of mucous membrane, and found that the glandular epithelial cells proliferate upwards to the surface, which they cover with flattened cells. These flattened cells grow out, and cover the whole raw surface by the 8th to the 10th day. The surface cells become cubical, and from them grow down tubes forming the new glands. Till the 30th day the glands are formed of cubical cells; at this time "pepsin cells" become differentiated at the base of the glands, and till the 55th day the glands increase in numbers. They make no mention of the development of oxyntic cells, but from a consideration of the figures in their paper I gather that they mean oxyntic cells by the term "pepsin cells." The above changes only occur at the stated times if the animal eat no food for four or five days at the beginning, and be then put on milk, and finally milk and bread for a time. If it be allowed to eat solid food at the beginning, regeneration has hardly commenced by the 8th to the 12th day.

The ulcers do not invariably heal in this rapid fashion, and in one case I found an unhealed ulcer on the 21st day. The microscopic investigation of this ulcer shows that its base is formed of dense fibrous tissue, which passes out on each side and blends with the muscular coats of the stomach. The edge of the mucous membrane on one side is recurved, but not on the other. At both edges the mucous membrane has grown out for a short space in a single layer of cells, and there ends at the edge of the ulcer.

The granulation tissue of the floor of the ulcer has fungated above the level of the regenerated mucous membrane, and this partially organised granulation tissue, for a depth of one-sixth of the whole thickness of the wall of the stomach, has undergone necrosis, the necrotic tissue extending up to the edge of the growing epithelium, where it stops. It is impossible for the cells to grow over this dead tissue, and hence the delay in the time of healing. This animal was fed on the same diet as the other twenty.

I have microscopically examined only one scar as old as 55 days.

The mucous membrane in this specimen is regenerated as described by Griffini and Vassale, but no oxyntic cells are present except at the very edge next to the normal mucous membrane. The mouths of the glands in places are dilated, and the glands widely separated by interstitial tissue.

The base of the scar is formed of loose fibro-cellular tissue, embedded in which are seen isolated strands of muscular tissue (fig. 2).

In another specimen, 41 days old, the base of the scar is formed of dense fibrous tissue, with thin and atrophied muscular tissue between it and the peritoneum. The edges of the normal mucous membrane are recurved. The regenerated mucous membrane is thin and the glands short, implanted directly on the fibrous tissue, and all dilated and lined by cubical cells. The mouths of the ducts are very wide. The interstitial tissue is excessive in amount and separates the glands widely. There are no central nor oxyntic cells to be seen. At one edge the regenerated mucous membrane is connected with the normal mucous membrane by a single layer of cubical cells lying on the fibrous base of the scar. From the condition of the glands it seems as if this ulcer were delayed, but eventually healed up.

III. *The Production of Motor Insufficiency of the Stomach of the Cat.*

In speaking of the motor power of the stomach, I mean the ability of the muscular coats of the stomach to empty the contents into the duodenum Deficiency in this ability or motor insufficiency is seen clinically in different degrees. If the degree of motor insufficiency be judged by the size or capacity of the stomach, the more extreme conditions only will be observed, and the less extreme conditions, in which the size of the stomach is often not much increased, will be neglected. Increased capacity does not necessarily imply a pathological condition, for in health the stomach is an organ the size of which is subject to wide variations.

A very large stomach may not be a "dilated stomach" in the pathological sense. The whole question turns upon whether or not the muscular coat of the stomach is able to propel the food into the small intestine within the physiological limits of time, and the essential feature in motor insufficiency is a delayed expulsion of food from the stomach. The slighter grades depend upon a so-called atony of the muscular coat, and in such a condition the motor function may be so damaged that food is retained for 24 hours or more, or probably upon a temporary spasm of the pylorus in some cases; the higher grades, in which complete stagnation occurs, depend upon pyloric obstruction.

The commonest form of indigestion of food is probably chiefly associated with delay in the emptying of the stomach. It is such a condition that I have endeavoured to reproduce experimentally.

Method.—My method of estimating the presence and the degree of motor insufficiency existing is the same as that employed clinically, namely, that of finding out whether food remains in the stomach beyond a certain time, with the difference that clinically one passes the stomach tube. In these cases to be absolutely exact I kill the animal.

After a meal of 100 to 120 grammes of meat, the cat's stomach is usually empty in 12 hours. Cannon (7) found this by the employment of the X-rays after Bismuth meals; I have confirmed it by killing a series of animals at different times. So that if a cat be given a meal of 100 to 120 grammes of meat at 6 P.M. its stomach should be empty by 9 A.M. on the following morning; if not, it is the subject of motor insufficiency of the stomach.

The method of producing motor insufficiency which I employ is that of artificial pyloric stenosis.

The constricting band consists of a piece of rubber tubing as used for constricting the vena cava in my experiments on cardiac dropsy (8). The rubber tubing (¼ inch or less in length) is slit open longitudinally and a silk ligature passed round the outside of it; each free end of the ligature is made to pierce the rubber tubing from without in and again from within out, the ligature now appearing just outside the cut margin. When the ends of the ligature are tied the cut margins come together and the lumen of the tube is restored. By taking a tube of appropriate diameter a thin-walled vessel such as a vein may be constricted to any definite fraction of its diameter required. This cannot be done exactly in the case of the duodenum, because its walls are too thick and it is a contractile organ, so that I have been unable to measure definitely the amount of constriction. The duodena of different cats vary enormously in diameter: a small cat may have a large duodenum and, *vice versâ,* a large cat a small duodenum; one has therefore to keep a supply of different sizes of tubes ready at each operation. I have found this method completely successful, but since one cannot measure the amount of constriction the latter has to be guessed, with the result that it is impossible to be absolutely certain what will happen in any given case. If too tight the animal will die, if too slack complete compensation occurs, but in most cases some degree of motor insufficiency results, and after a little practice this end can be accomplished in the vast majority of cases. The abdomen is opened in the middle line under strict antiseptic precautions, and the rubber tubing applied just beyond the pylorus around the first part of the duodenum.

The animals with motor insufficiency have diminished appetite and lose flesh; they occasionally vomit, but, curiously enough, vomiting may be practically absent in the higher grades of retention of food, possibly due to depressed sensibility of the sensory nerves of the stomach. In the slighter grades there is a delay in emptying the stomach, and in these there may be slight dilatation of the stomach or not; at all events the muscular coat has diminished resistance to stretching after death. A curious feature in these cases is the amount of hair found in the stomach. Hair is always liable to be found in the cat's stomach, due to the animal's habit of licking. In cases of motor insufficiency the hair collects in the stomach, as apparently it is not so able as food to pass the pylorus. The meat is cut up for the animals, as they will not usually eat large pieces. The amount of the appetite is easily estimated by weighing everything the animal is given and everything it leaves. In the higher grades of insufficiency the stomach is usually found dilated and thinned, and may contain large quantities of brown acid fluid. On the other hand, the stomach may be found empty when vomiting has been a prominent symptom. In the latter cases retention of food is probably largely absent, as what remains is vomited. The animal may discover exactly how much its stomach is capable of dealing with within the physiological limits of time, so that no retention occurs. The presence of diminished appetite, vomiting, and wasting are therefore not definite indications of retention of food. The only tests which are of value are the presence of food in the stomach a certain length of time after a test meal, or the presence of dilatation of the stomach.

IV. *The Effects of Motor Insufficiency upon the Healing of Acute Gastric Ulcer in the Cat.*

The pylorus is first constricted and, after the animal has recovered and settled down to a more or less definite diet, a gastric ulcer is produced as described above on the anterior wall of the stomach, midway between the cardiac and pyloric orifices.

There are four groups of experiments.

Group I. Six experiments:—

Cat 1: weight 2865 grammes.

February 4, 1909.—Pylorus constricted. Tube, 9 mm. Vomited twice; appetite soon recovered and in a few days eats an average of 250 grammes daily; no vomiting.

February 19.—Intramural injection of 6 *c.c. serum.*

March 11.—Test meal, 120 grammes meat at 6 P.M., all eaten. Weight 3105 grammes.

March 12.—Killed 9 A.M.; stomach empty except for considerable amount of hair. Acid in reaction. Scar of healed ulcer present (21*st day*) (fig. 3, A). This animal is useful as a control, as no motor insufficiency was present.

Cat 2: died the *day after* the serum was injected.

Cat 3: weight 3700 grammes. February 4, 1909.—Pylorus constricted. Tube, 9 mm. Vomited once or twice; appetite recovered, and in a few days eats 100 to 150 grammes. Weight 2895 grammes.

February 19.—Intramural injection of 6 *c.c. serum.* Appetite lost at first, but in a few days recovered to the extent of about 130 grammes a day, and then gradually lost again, and finally eats 10 to 30 grammes a day only; no vomiting.

March 11.—Test meal, 6 P.M., not eaten. Weight 2500 grammes.

" 12.—Killed 9 A.M., stomach much dilated, wall thin and it maintains its shape on opening. Contains 160 c.c. brownish mucoid fluid containing 0·259 per cent. HCl. Large ulcer healing, base somewhat thickened and composed of granulation tissue on free surface (21*st day*).

Cat 4: died on the *eighth day* after the injection; 20 c.c. yellowish acid fluid in stomach, which was not dilated. An ulcer was present about the same size as that of Cat 6 in a sloughing condition.

Cat 5: weight 3610 grammes. February 10, 1909.—Pyloric constriction. Tube, 9 mm. Soon recovered and appetite good. Able to eat 200 grammes a day.

February 19.—Intramural injection of 6 *c.c. serum.* Appetite never bad, easily eats 200 grammes. Vomited once.

March 25.—Test meal, 105 grammes, 6 P.M. Weight 4270 grammes.

March 26.—Killed 9 A.M. Stomach distended and contained 80 grammes meat and some hair. An unhealed ulcer present, the base being formed of granulation tissue with a little hæmorrhage from it. Base thickened and omentum adherent to it. Feels like a thick nodule of fibrous tissue in the stomach wall (35*th day*) (fig. 3, B).

Cat 6: Died on the *third day* after injection; a large perforation present.

Group II. Eight experiments.—The pylorus was constricted in each animal, and on April 28, 1909, 8 *c.c. immune serum* of the goat were injected into the stomach wall of each. Six of these animals died within 10 days; of these, three had large ulcers, two perforated, and one had a large ulcer with extensive hæmorrhagic infiltration and ulceration of the mucous membrane around.

This extensive ulceration, with or without hæmorrhagic infiltration spreading round the ulcer, I have found in six cases of pyloric stenosis, and never apart from it. In all the cases the fluid in the stomach was neutral or alkaline. Occasionally there is some œdema of the stomach wall. It is possible that this condition may be due to a secondary bacterial infection owing to the alkalinity of the stomach contents, but this point remains to be investigated. The stomach contents, however, may be alkaline in the absence of this spreading ulceration.

Of the two remaining animals, one died on the 26th day with a large unhealed ulcer and the stomach a little dilated and thinned, and the other survived and was killed on the 56th day. The stomach of the latter animal was not dilated and it had disposed of its test meal of 60 grammes in 15 hours. There was a large triangular depressed scar with some contraction of the stomach at that spot. *Microscopical examination*: The base of the original ulcer is formed of young fibrous tissue composed of elongated cells and short fibres interlacing in every direction and packed together into a dense mass. The newly formed mucous membrane is directly implanted on this fibrous tissue. There is no recurving of the normal mucous membrane. The newly formed glands are largely made up of cubical or columnar epithelial cells, but at the base in many places central cells have been formed, but no oxyntic cells. The glands mostly have distinct lumina and a large number of cystic spaces has been formed, the cysts being lined by flattened cells. The mouths of the glands are wide open and in many places a coarsely villous appearance is given to the surface (fig. 4). A great deal of cellular infiltration exists between the glands and in places projections of fibrous tissue from the base cut up the mucous membrane. In some of the large cysts ridges of fibrous tissue covered with epithelium project into the lumina, partially dividing them into locculi. Of the controls, two died with large ulcers during the first week and the remaining one was killed on the 55th day, showing the usual radiating scar of normal healing.

Group III. Seven experiments.—The pylorus was constricted in each animal, and on May 14, 1909, 6 *c.c. immune serum* of the goat were injected into the stomach wall of each. Six of these animals died within five days; of these, four had hæmorrhage infiltration and ulceration around the ulcer, which had been produced, and in one of these cases the ulceration occupied half the whole area of the mucous membrane of the stomach. In each case the fluid in the stomach was alkaline or neutral. Of the remaining two, one perforated and the other had an ulcer of the usual size. One of the animals survived and was killed on the 41st day. There was a little dilatation, and a small unhealed ulcer was present. This was to some extent concealed by the surrounding mucous membrane, which was very exuberant. *Microscopical Examination*: The edges of the normal mucous membrane are turned in, the mouths of the glands almost touching the thin epithelium covering the healed portion of the ulcer. The whole thickness of the stomach wall at this spot is composed of dense, sclerotic, fibrous tissue, with round cells in spaces here and there. In the centre is an unhealed portion occupying about one-fourth of the diameter of the original ulcer. The base of this unhealed portion is formed of looser tissue, containing cells and a few dilated capillary vessels, and in the centre this tissue projects and has undergone necrosis. The base of the original ulcer is much thicker in the centre than at the sides. There is another unhealed portion towards the side, about one-fourth the size of the former, where the recurved mucous membrane touches the base of the ulcer. The reformed epithelium covering the healed portion consists of a single layer of cells, apparently a continuation of that covering the surface of the normal epithelium. The cells are columnar in type, and as they near the edges of the two ulcerated surfaces gradually become flatter and flatter till they look like endothelial cells and disappear (fig. 5). The cells are implanted directly upon the fibrous tissue of the base. At one spot two tiny gland ducts have started to grow, each consisting of a dozen or more cells and forming little depressions in the fibrous tissue, otherwise the reformed epithelium consists merely of a single layer of cells.

Of the control animals, one perforated on the 10th day and the other was killed on the 41st day and showed a small triangular scar in the stomach.

Group IV. Eight experiments.—The pylorus was constricted in each animal and on June 4, 1909, 5 *c.c. immune serum* of the goat were injected into the stomach wall of each. Four of these animals died within the first 15 days. Of these, one perforated, one had hæmorrhagic infiltration and ulceration around the ulcer, and two had ulcers of the usual size.

The remaining four cats survived and all had the scars of healed ulcers. Of these, two must be excluded because the stomach had been able to compensate and no motor insufficiency developed. The remaining two, whose autopsies were performed on the 52nd and 55th days respectively, and which had motor insufficiency, remain to be considered.

52nd Day Cat.—The base of the scar is formed of dense fibro-cellular tissue, at the outer part covered with a thin layer of atrophied muscle. The edges of the normal mucous membrane are recurved and where they press on the base of the scar have flattened the newly formed epithelium so that at one spot it only consists of a single layer of cubical cells. The new epithelium covering the scar has grown up into glands in the centre, where it is freed from pressure. The glands are formed entirely of duct epithelium directly implanted on the fibro-cellular tissue. There are no central nor oxyntic cells present. There is a good deal of cystic dilatation at one side where the normal joins the new mucous membrane. These cysts are lined by flattened epithelium. There is a considerable amount of new connective tissue between the glands.

55th Day Cat.—The base of the scar is not formed of such dense fibrous tissue as in the former cat, and the muscular coats of the stomach have not been destroyed to the same extent. The mucous membrane is thicker and the glands fairly well formed, central cells are present at the base and in places oxyntic cells can be seen. The glands are of irregular shapes and separated by infiltration of new connective tissue. There is some cystic formation of the glands.

The two control animals both lived and showed the radiating scars of normal healing on the 55th day. Sections of one showed the mucous membrane completely reformed as I related in describing the normal healing of acute gastric ulcer.

In *Group I*, Experiments 1, 3, and 5 are conclusive. In Experiment 1 there was no motor insufficiency and the ulcer healed in the usual way, leaving a small stellate scar, within 21 days. In Experiments 3 and 5 there was motor insufficiency and the ulcers were unhealed on the 21st and 35th days respectively. Experiments 2, 4, and 6 are of use as controls to show what the ulcers were like in the early stages.

In *Group II* the 26th day animal had motor insufficiency and an unhealed ulcer. The 56th day animal showed a scar with considerable fibro-cellular thickening and imperfectly formed glands. The fibrous thickening and imperfect glands show a delay in the healing, but I have not included this case because I was unable to prove that there was motor insufficiency, the animal having 15 hours in which to dispose of its 60 grammes of food, although I think that it was present.

In *Group III* the 41st day animal had an unhealed ulcer and motor insufficiency. There was dense fibrous thickening of the sclerotic type and the mucous membrane which was reformed consisted of a single layer of cells such as should be present on the 8th or 10th day of normal healing according to Griffini and Vassale.

In *Group IV* the 52nd day cat had a scar of considerable fibro-cellular thickening covered with a mucous membrane composed of very imperfectly formed glands such as should be present on the 16th day of normal healing. The 55th day cat had a scar the base of which was not so dense, and the glands were almost completely reformed.

It is quite evident from these experiments that retention of food produced by motor insufficiency may delay the healing of an acute gastric ulcer for a period at least twice the length of the normal. Different degrees of insufficiency produce differences in the amount of delay.

The delay occurs at two stages:—

(1) When a single layer of epithelium covers the base of the ulcer and before glandular formation has commenced (= 10th day of normal healing).

(2) When the glands have been reformed but the central and oxyntic cells not differentiated. The glands are merely formed of duct epithelium at this stage (= 16th day of normal healing).

Whether, if the animals had been allowed to live, completely formed glands would have eventually developed is a very interesting question. In the normal healing of an ulcer there may be some delay, as is seen from the two cases quoted above, in which one was delayed owing to necrosis of the granulation tissue at the base of the ulcer, and the other was healed, but covered with glands of the duct epithelium type. So that the delay occurring in cases of motor insufficiency is merely an exaggeration of the delay which may occur in exceptional cases normally. That it is a true delay is obvious, because the different conditions found exactly correspond to the various stages in the normal healing of an ulcer.

Cause of the Delay in Healing.—For the normal regeneration of the mucous membrane to take place it is necessary (1) that the epithelial cells should be uninjured and free to grow over the base of the ulcer; and (2) that the base of the ulcer should consist of healthy granulation tissue containing an abundance of cells, so that the over-growing epithelial cells can be properly nourished, and so that the tissue over which the cells grow can supply a suitable stroma for the growth of the glands from the surface epithelium. If either of these conditions be not fulfilled the healing must be delayed.

It will, I think, be generally admitted that the epithelial cells are more resistant to the action of the gastric juice than the tissue forming the base of the ulcer, and the great resistance displayed by these cells is seen in the promptitude with which they cover the surface, and by the fact that they can resist the action of HCl of 0·7 to 0·9 per cent. strength (4). Of course, the incurving of the mucous membrane at the edges of the ulcer must offer a formidable resistance to the growth of the cells, both by the abnormal direction in which the cells have to grow, and the pressure exerted on the base of the ulcer by the incurved mucous membrane. Still, this is by no means an insuperable obstacle, as the cells are readily able to grow round the angle in a single layer and, when free from the pressure, to sprout out into glands in the centre of the ulcer. So that one is rather compelled to look for any conditions which may be present in the *base of the ulcer* which can prevent the epithelium growing over it or are able to modify its growth. If the base of the ulcer be necrotic, as may occur as the result of bacterial invasion or digestion by the gastric juice, it is obvious that the growth of the epithelium over it would be at once arrested; and, on the other hand, if the base be irritated and dense fibrous tissue rapidly formed, it is equally obvious that the epithelium would be modified in its growth, both by deficient blood supply and the failure of the fibrous tissue to form a suitable stroma for the process of glandular formation. In the three cases described in the normal healing of ulcer, I have mentioned that in one the exuberant granulations had become necrotic and had stopped the growth of the epithelium; that in another the base was formed of dense fibrous tissue and the overlying glands formed of duct epithelium only; and that in the third the glands were completely regenerated and the underlying tissue more loose and cellular in character.

In the cases of pyloric stenosis the same principle is observed. In proportion as the base is sclerotic the more difficult is it for the epithelium to be regenerated and the glands to proliferate. It follows from this that *the delay in the healing of an acute gastric ulcer is not so much due to a fault in the epithelium as to the condition of the base of the ulcer over which it is growing.*

It was shown by Griffini and Vassale, as stated above, that the base of a defect in the mucous membrane is covered with surface epithelium in 8 to 10 days if the animal eat no food for 4 to 5 days and is then put on milk, but that regeneration of the epithelium has hardly commenced by the 8th to 12th day if it eat solid food from the first day. The condition of the gastric contents is therefore of supreme importance in the healing of ulcer. In pyloric stenosis, food saturated with gastric juice is retained beyond the normal time. Excessive irritation and injury of the base of the ulcer results and bacteria have more time in which to attach themselves to it, so that in the early stages excessive exudation of leucocytes and perhaps necrosis of the granulation tissue results, and in the later stages excessive fibrosis. The former condition will delay the growth of the surface epithelium over the base, and the latter will prevent the regeneration of the glands. This appears to me to be comparable to the failure of cancer to grow in an immune mouse, the latter being unable to furnish a vascular stroma for the cancer to develop in (9). It is conceivable that excessive formation of sclerotic tissue in the base of the ulcer might completely prevent the growth of epithelium over it in which case the ulcer would deepen from digestion of the fibrous tissue, but of this I have no actual proof. Simple motor insufficiency will *delay* the healing of an acute ulcer, but will not *stop* the healing nor *make the ulcer extend.*

8. *Effect of Motor Insufficiency upon the Size of the Ulcer.*—A comparison of the sizes and tendency to perforate of the ulcers produced in the above cases of pyloric stenosis with those of the control animals shows that motor insufficiency has no influence in increasing the sizes or tendency to perforate of the ulcers produced.

On the other hand, in 6 out of 29 cases there were considerable hæmorrhage and acute ulceration around the spot where the ulcer was produced, and in all these cases the gastric contents were alkaline or neutral. This is undoubtedly a direct result of the pyloric stenosis, as I have never seen it apart from that condition. Whether or not it is due to a secondary bacterial invasion of the ulcer, I am not at present in a position to state.

V. *Conclusions.*

1. A gastrotoxic serum active against the cat may be prepared by immunising the goat with cat's gastric cells.

Its properties, which have been examined, have been found to correspond to those of the gastrotoxin formed by immunising the rabbit with guinea-pig's gastric cells.

2. Acute gastric ulcer in the cat heals within a few weeks, as in the case of the guinea-pig. This result agrees with those of other observers who have produced lesions by injury of the gastric mucous membrane of dogs.

3. The ulceration produced by gastrotoxin is more extensive if produced whilst the stomach is digesting than whilst it is resting; in the latter case ulceration may fail to appear.

4. Motor insufficiency of the stomach definitely delays the healing of gastric ulcer for at least twice the normal time. There is more thickening of the base and less complete regeneration of the gastric glands than occurs in cases of normal healing. This may explain the beneficial effect following gastro-enterostomy for chronic gastric ulcer in man.

5. The character of the mucous membrane covering the base of an ulcer, in which the healing has been delayed, varies in proportion to the delay. It may consist of a single layer of epithelial cells on the 41st day, such as should be found on the 8th to the 10th day of normal healing; of regenerated glands consisting merely of duct epithelium on the 52nd day, such as should be found on the 16th day; or of almost completely regenerated glands. Whether eventually in process of time the glands would be always completely reformed has not been determined, but more likely they would not.

6. The delay in the healing in cases of motor insufficiency is due to a fault in the base of the ulcer, such as may occasionally be seen in normal healing. This fault may be due to necrosis of the base of the ulcer or excessive formation of sclerotic tissue therein, such conditions being the result of the low resistance which the connective tissues possess to digestion by the gastric juice, or possibly in some cases to a secondary bacterial infection.

7. When pyloric stenosis is present, extensive ulceration may be found around the ulcer, probably due to a bacterial infection. The ulcer actually produced by the serum, however, is no more liable to perforate nor to be more extensive than in the control animal.

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DESCRIPTION OF PLATES.

PLATE 8.

FIG. 1.—To illustrate the normal healing of gastric ulcer in the cat.

A. Fourth day.—A punched out ulcer, with cleanly cut edges, extends through the muscular coat of the stomach.

B. 16th day.—This specimen shows an excessive amount of contraction, which is unusual. In the centre is a small unhealed surface.

FIG. 2.—Section of cat's stomach showing regeneration of the glands on the 55th day. The glands are irregular, no oxyntic cells are present, and there is a considerable amount of interstitial tissue.

PLATE 9.

FIG. 3.—To illustrate the delay in healing produced by motor insufficiency of the stomach.

A. Stomach of control cat, Group 1. (Pyloric stenosis.) A small radiating scar is seen (21st day) to the right of the centre.

B. Stomach of Cat 5, Group 1. An unhealed and thickened ulcer is present (35th day). The stomach is a little dilated and the rugæ are not so prominent as normal.

FIG. 4.—Section of stomach of 56th day cat, Group 2. (Pyloric stenosis), showing very irregular growth and cystic dilatation of the regenerated glands.

FIG. 5.—Section of stomach of 41st day cat, Group 3. (Pyloric stenosis), showing an unhealed ulcer with sclerotic base and the epithelium growing over it in a single layer of cells.

On the Action of Antimony Compounds in Trypanosomiasis in Rats: being a Report to a Sub-Committee of the Tropical Diseases Committee of the Royal Society.

By John D. Thomson, M.B., C.M., and Arthur R. Cushny, M.D., F.R.S.

(Received November 23, 1909,—Read January 20, 1910.)

The near chemical and pharmacological relation of arsenic and antimony suggested naturally the use of the latter in a disease in which the former had proved of value; but the irritant action of the ordinary antimony salts seemed to preclude their use by hypodermic injection. After repeated attempts to form organic compounds of antimony analogous to atoxyl, one of us (C.) submitted to Plimmer and Thomson, for experimental trial, a compound of glycine and antimony, and their results with it showed that antimony possesses trypanocidal properties. This first antimony compound proved difficult to make and unstable, however, and these observers substituted for it tartar emetic; the results were satisfactory, and the following investigation was undertaken with the object of determining the limits of usefulness of other antimony compounds in these diseases. The experiments were made on rats infected with a strain of Nagana (*T. brucei*) obtained by the kindness of Sir J. McFadyean. This strain was fatal to rats within six days after inoculation, or within three days after trypanosomes appeared in the blood. The inoculation and treatment were carried out at the Lister Institute of Preventive Medicine.

In the use of the heavy metals it is generally recognised that the more readily dissociated compounds are liable to cause more local irritation than others, and where the local action is to be avoided, attempts are made to use compounds which are less immediately dissociated and pass into the general tissues in their original form, there to free the metallic ion compound which is necessary for the desired effect.

An example of this is offered in the substitution of atoxyl for the older arsenic preparations in the treatment of trypanosomiasis, for there is no question that the atoxyl owes its activity to the simpler compounds formed from it in the tissues. It is possible that, in addition to avoiding local irritation, such compounds as atoxyl may penetrate into cells which are not permeable by ordinary arsenic salts, and that the latter may in this way be liberated in cells to which they would not otherwise have had access.

With this in mind it seemed desirable to test the action of compounds in which antimony is more firmly combined than the ordinary salts, but very few organic combinations are available, and we have succeeded in obtaining only two such for investigation. Of these the first was tetraethylstibonium iodide ($(C_2H_5)_4SbI$), which was injected into seven Nagana rats in quantities up to 20 milligrammes, but had no effect whatever on the parasites, the injected animals dying at the usual time, and the blood being found to be swarming with trypanosomes. The other, diphenylstibinchloride ($(C_6H_5)_2SbCl_3$, H_2O), was kindly put at our disposal by Prof. A. Michaelis, of Rostock, but proved quite devoid of action in quantities of 4 c.c. of a saturated solution in sodium carbonate, in which it is more soluble than in water. These compounds appear non-irritant, but the antimony is probably not freed in the tissues, the first compound in particular resembling the ammonium salts, which pass through the animal body without freeing nitrogen.

These compounds proving valueless for our purpose, it was determined to find in what form the antimony ion had to be liberated to be effective, and a number of compounds commercially obtainable were tested. Of these the potassium metantimoniate (SbO_2OK), injected in quantities up to 30 milligrammes, scarcely affected the trypanosomes in the blood; doses above 30 milligrammes were followed by the disappearance of the parasites from the circulation, but the rats became very ill and died within a few days, and *post mortem* enteritis and nephritis were found to have been developed. A preparation of antimony oxide (Sb_2O_3), stated to be in colloidal form, was obtained from Kalle and Co., but proved extremely irritating, and had a very low efficiency. The preparation contained 4·5 per cent. of antimony oxide only, and in quantities corresponding to 4 milligrammes of the oxide had little, if any, effect in reducing the number of trypanosomes in the blood; 8 milligrammes caused their disappearance, but they recurred on the fourth day.

In the former of these preparations antimony is presented in pentavalent, in the latter in trivalent, form, and both are equally inefficient, while in the forms in which it is presented in combination with organic acids it is also in trivalent form, but has been shown to have a high degree of activity. It is possible that, given in the colloid form, it is deposited locally and fails to reach the trypanosomes. This would also explain the intense local action, which was more marked from this than from any other preparation.

A glyceride of antimony, analogous to boroglyceride, was formed by heating glycerine with the oxide, and proved fairly effective in destroying the trypanosomes in the rats, but it was very irritant and caused hæmoglobinuria, and the solution in glycerine tended to deposit the oxide when diluted.

A few experiments were made with Schlippe's salt, sodium sulphantimonate ($(NaS)_3SbS$); it destroyed the trypanosomes in the rat very satisfactorily (see Table I), but induced very considerable local reaction, and therefore appears to be precluded from use in therapeutics. This result agrees with that obtained by Broden and Rodhain* in man.

Table I.—Summary of Results of Treatment with Schlippe's Salt.

I.	II.	III.	IV.
Number of rats treated.	Number of rats that died without recurrence, but before any deduction could be made.	Number of rats surviving over one month after cessation of treatment without recurrence.	Number of rats in which recurrence occurred.
20	6	3	11

Of the rats included in Column III: One survived 39 days, one 33 days and one 76 days. The cause of death did not seem to be the disease or its treatment.

Of the rats included in Column IV: Recurrence took place in 10, 21, 9, 10, 10, 13, 7, 13, 16, and 14 days, and all died within two weeks of the cessation of treatment from the recurrence (or from the last recurrence, for many had more than one recurrence), with the exception of one which was alive and well 244 days after cessation of treatment of the recurrence—the latter by another drug (sodium antimonyl tartrate).

In Schlippe's salt and in the glyceride, antimony is pentavalent, and these were efficient trypanocides, especially the former, while the metantimoniate, where antimony is also pentavalent, possessed a low efficiency.

The sulphantimoniate differs from the metantimoniate in its great instability, and it seems probable that the marked local reaction arising from it is due in great part to its being decomposed at the point of injection with the deposit of the insoluble antimony sulphide Sb_2S_3, which produces a slow, lasting reaction. Enough reaches the general tissues, however, to react with the parasites, and here its instability, permitting of its forming new compounds, renders it peculiarly active. The metantimoniate, on the other hand, is much more stable, and probably fails to be reduced to the trivalent form.

The greater number of our experiments were done with the combinations of antimony with the organic acids corresponding to the ordinary tartar emetic. Among these the best combinations were found to be those with oxyacids of the fatty series, those of the aromatic series proving much less soluble. Among the salts examined were the lactate, citrate, malate, and mucate, the results of which were compared with those obtained by the tartrate.

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* 'Arch. f. Schiffs- u. Tropenhygiene,' vol. 12, No. 14, 1908.

They were formed by boiling antimony oxide with the acids, and subsequently neutralising with sodium hydrate, or sometimes by forming the acid sodium salt, and boiling it with freshly prepared antimony oxide. The mucate, which was investigated with the idea that its multiple hydroxyl groups might prove to have special powers of retaining antimony in solution, may be dismissed, as, though powerfully trypanocidal, it induced very great local irritation. The citrate seemed to be inferior to the malates and tartrates, which were approximately equal in value.

Table II.—Summary of Results of Treatment with Sodium Antimonyl Malate.

I.	II.	III.	IV.
Number of rats treated.	Number of rats that died without recurrence, but before any deduction could be made.	Number of rats surviving one month after cessation of treatment without recurrence.	Number of rats in which there was recurrence.
23	9	5	9

Of the rats included in Column III: One survived 241 days; one 113 days; one 115 days; one 66 days; and one was alive and well, 235 days after cessation of treatment.

Of the rats included in Column IV: Recurrence took place in 12, 11, 7, 17, 5, 11, 9, 36, and 10 days, and all died within two weeks of cessation of treatment of recurrence (or of the last recurrence), except one which lived 231 days after cessation of treatment of the recurrence by another drug (Schlippe's salt).

Among the tartrates and malates, the sodium and potassium salts were equally efficient trypanocides, and there was no appreciable difference in their local effects, but the substitution of an alkyl radical for the potassium or sodium of the salt seemed to be attended with some advantage. Solutions of ethylantimonyl tartrate were kindly prepared for us by Prof. Collie by heating freshly precipitated antimony oxide with ethyl tartrate, to about 150° C. in sealed tubes. The solutions are distinctly acid, but can be neutralised or rendered slightly alkaline with ammonia, and can be sterilised by boiling without any cloudiness resulting. It is a very efficient trypanocide and causes no local irritation in the rat. After the injection of 1 c.c. of a 0·2-per-cent. solution* into rats of 100 to 200 grammes weight, trypanosomes, though previously numerous, entirely disappear from the peripheral blood within one to two hours. Estimated by the amount of antimony present in the solutions, the ethyl is more powerfully trypanocidal than the sodium salt, which may suggest either that less is deposited at the point of application, or that it reaches the trypanosomes in a more readily penetrating form.

Though more poisonous to the rat than sodium antimonyl tartrate, the *range* of dose, or the difference between the effective trypanocidal dose and the lethal dose, is not less than that of the other antimony preparations. The optimum dose may be found to correspond to Browning's therapeutic dose, viz.: two-thirds of the maximum dose that average animals tolerate.

From our own work, and that of others, we think that sufficient data have been obtained to indicate some points which must be taken into consideration in attempting further advance in the treatment of trypanosomiasis :—

(1) As regards the compound it must be non-irritant and capable of remaining in perfect solution at the temperature and alkalinity of the tissues.

(2) It must act quickly on the trypanosomes, for otherwise these parasites seem to acquire a tolerance to it. It is possible that some drugs may require to be altered in the tissues before they begin to affect the parasites; but with this proviso, we suggest, as a working rule, that no drug which, given in full therapeutic dose, does not destroy the trypanosomes in the blood within two hours is likely to prove an advance on remedies already in use.

(3) When the trypanosomes have been expelled from the blood by a single full therapeutic dose, there must be no recurrence in the majority of cases within some fixed time, which will vary with the particular host, and the species and strain of trypanosome in use; the length of this period must be determined by each investigator by reference to some of the known trypanocides. In our experiments it proved waste of time to persevere with any drug whose administration in a single full dose was followed by a recurrence in the majority of cases within a week. The longer the time during which there is no recurrence in the majority of animals treated the more promising is the outlook for the drug under trial. But the non-recurrence in a single individual is of comparatively small importance.

In our experiments the majority of the rats that survived the injection for three weeks showed recurrence within that time, except when ethyl antimonyl tartrate was employed, when the results were slightly more favourable.

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* The strength of the solution was ascertained by estimation of the antimony.

In the recent report* from Uganda, the conclusion is drawn that the medicinal treatment pursued up to that time had proved of little lasting benefit in the great majority of even the early cases of Sleeping Sickness. From this it seems a fair inference that remedies which suffice to change an acute trypanosomiasis into a more chronic form may ameliorate symptoms, but do not necessarily greatly prolong the natural course of a chronic infection such as Sleeping Sickness. In seeking for a remedy for the chronic condition, by experiments with acute infections, nothing short of an immediate and complete disinfection should be the object, and it is with this view that we venture to suggest the foregoing considerations.

Applying these principles to the ethyl antimony compound, it appears to comply with the first fairly satisfactorily. It expelled the trypanosomes from the blood within two hours. As regards recurrence after a single full dose, of 13 rats inoculated, 6 showed a recurrence, on the 14th, 16th, 16th, 22nd, 26th, and 29th day. There was no recurrence in the 7 others.

Table III.—Summary of Results of Treatment with Ethylantimonyl Tartrate.

I.	II.	III.	IV.
Number of rats treated.	Number of rats that died without recurrence, but before any deduction could be made.	Number of rats surviving over a month after treatment without recurrence.	Number of rats in which there was recurrence.
13	0	7	6

Of the rats included in Column III, one died of pneumonia on the 84th day, and five of the others from exposure to cold between the 135th and 165th day, leaving one survivor after 260 days.

Of the rats in Column IV, recurrence took place after 14, 16, 16, 22, 26, and 29 days respectively.

No other drug has given such favourable results in our experiments, but we recognise that it will be necessary to test it against other species and strains and in different hosts before any general statement as to its usefulness can be made.

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* Quarterly Report on the Progress of Segregation Camps and Medical Treatment of Sleeping Sickness in Uganda (Quarter December 1, 1907, to February 29, 1908).

We have also treated some rats with a solution combining the best of the arsenical and antimonial trypanocides at present known to us, viz., ethylantimonyl tartrate and atoxyl. Twelve rats were inoculated with the Nagana strain, and on the third day after, when a fair number of trypanosomes were present in the blood, they received a single injection of 0·75 c.c. of a solution containing 2 per cent. of atoxyl dissolved in 0·2 per cent. of ethyl antimonyl tartrate.

Table IV.—Summary of Results of Treatment with Atoxyl + Ethylantimonyl Tartrate.

I.	II.	III.	IV.
Number of rats treated.	Number of rats that died without recurrence, but before any deduction could be made.	Number of rats surviving over a month after treatment without recurrence.	Number of cases of recurrence.
12	2	8	2

Of the rats included under Column III, two died in 31, one each in 39, 43, 56, 89 days, and two survived after 260 days. Thus, while the number of recurrences was fewer than from the antimony alone, the combination of the two drugs seemed to tend to be toxic. This was more obvious in a series of 19 rats treated with a single injection of 0·75 c.c. of 3-per-cent. atoxyl in 0·3-per-cent. ethylantimonyl tartrate solution. Of these, 11 died within 24 hours of the injection, and as the blood was free from trypanosomes, they evidently succumbed to the treatment.

Table V.—Summary of Results of Treatment with Larger Doses of Atoxyl and Antimony.

I.	II.	III.	IV.
Number of rats treated.	Number of rats that died without recurrence, but too soon for any deduction.	Number surviving over a month without recurrence.	Number of cases of recurrence.
19	16	2	1

Of the two rats included in Column III, one died on the 113th day, and one survived after 225 days. The recurrence noted in Column IV was noted on the 25th day. One of those in Column II had trypanosomes in the blood the day after treatment and died that day.

The results of the combined medication were thus scarcely superior to those of the ethylantimonyl tartrate alone, for though there were fewer recurrences when atoxyl was added, the mortality from poisoning was higher, so that only two survived for two months as against six of those treated with antimony alone. It is possible that by a more accurate adjustment of the two drugs the advantages of diminished recurrence and low toxicity might be combined.

Amakebe: A Disease of Calves in Uganda.

By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. Sleeping Sickness Commission of the Royal Society, 1908—09.

(Received December 18, 1909,—Read January 20, 1910.)

[Plate 10.]

Introductory.

Amakebe is the most important disease of cattle in Uganda. It attacks the calves soon after they are born, and destroys more than half of them. Among the native cattle the loss is reported to be as much as 75 per cent., but, with careful nursing and hand-feeding, this mortality may be reduced to between 20 and 30 per cent. This is an enormous toll to pay, and renders the breeding of cattle in Uganda for dairy purposes, or, indeed, for any purpose, very up-hill work.

Little up to the present has been written as to the nature and causation of Amakebe. It has been described as a trypanosome disease, but this evidently on insufficient knowledge.

Distribution in Uganda.

Amakebe appears to occur all over the Kingdoms of Uganda, Unyoro, Ankole, and Busoga. Lieutenant A. D. Fraser, Royal Army Medical Corps, the medical officer lately in charge of the Sleeping Sickness Camp, Sesse, reports, however, the curious fact that it does not occur among the cattle on the Sesse Islands. Mr. C. W. Hattersley also informs the Commission that cows brought to Mengo from Ankole invariably contract the disease, which would go to show that in some parts of Ankole the disease does not occur. Mr. R. J. Stordy, the chief veterinary officer, British East Africa, states that Amakebe is found at every altitude in that Protectorate. Dr. A. Theiler, C.M.G., the chief veterinary bacteriologist, Transvaal, who lately visited Uganda, writes that Dr. Lichtenfeld, the principal veterinary officer, German East Africa, told him that a disease similar to Amakebe exists in Ruanda, on the western shores of Victoria Nyanza and adjoining Ankole.

It is evident, then, that this disease is widely prevalent in Central Africa, and most disastrous in its effects.

Nomenclature.

In Uganda the disease is known as "kebe," "makebe," or "amakebe," and means calves' swollen glands, or mumps. At Ngora, to the west of Mount Elgon, the natives call the disease "angarwe." In Unyoro, "masugu." In Ankole, "amashuyu" or "amashui."

Symptoms.

The chief symptom of this disease is the swelling of the lymphatic glands, especially those in the region of the ear, in front of the shoulder, and in front of the hip. The glands frequently reach a large size, those in front of the shoulder often being three or four inches in length. They are soft to the touch, giving the impression of an elastic body under the skin. The hair is rough and staring, the head hangs, the ears droop, and there is frequently a watery discharge from the eyes and nose. During the illness the temperature runs high, often reaching 107° F. or more. The calf becomes rapidly emaciated, and often a dry, scabby eruption of the skin is seen. Diarrhœa is frequent, and the dung is often dark in colour, with an evil odour. The urine never shows any trace of blood, as in redwater.

The duration of the disease is usually about a fortnight, but sometimes the calves get over it in three or four days. The fever goes, they pick up condition, and the swelling of the glands subsides. The glands, however, never regain their normal size, but remain permanently enlarged throughout life.

When a calf has recovered from Amakebe it is no longer susceptible to the disease. It is immune for the rest of its life.

The following cases illustrate the course of the disease :—

Experiment 1387.—To study Amakebe in the Calf.

July 26, 1909.—Animal received from Sir Apolo Kagwa, K.C.M.G., Kampala.

July 29.—The prescapular glands are the size of a walnut. The calf looks fairly well, is thin, and hair slightly rough.

Aug. 2.—The lymphatic glands are much more enlarged. The prescapular glands measure 3½ by 2 inches.

„ 12.—This calf is now looking very sick. Conjunctival mucous membrane congested. The hair is falling off in patches, leaving a rough, scabby surface. Diarrhœa.

„ 14.—Discharge from eyes and nose. Diarrhœa.

„ 26.—This calf got steadily worse, and died at 11.30 A.M. to-day.

The following chart represents the course of the temperature:—

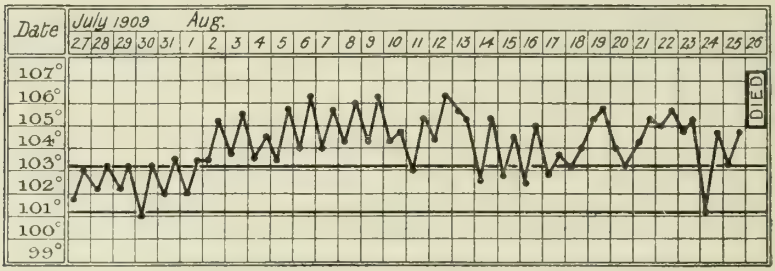

CHART 1.—Experiment 1387. Temperature Curve in a severe Case of Amakebe.

Aug. 26, 1909.—*Post-mortem* immediately after death.

External Appearances.—The body is emaciated. *Rigor mortis* absent. The hair is staring and has a ragged appearance. There are many patches of eruption on the surface of the body, especially on the face and head. These eruptions are, as a rule, about the size of half-a-crown and resemble limpet-shells.

Internal Appearances.—On removing the skin, the flesh is seen to be pale in colour. On opening into the abdomen the spleen is seen to be enlarged. The surface of the stomach and intestines is exceedingly pale and yellowish. There is no fluid in the peritoneal cavity. On opening into the thorax no fluid is found in either pleural cavity. There is about a tablespoonful of bright, chrome-coloured, clear fluid in the pericardium. The anterior mediastinum contains a quantity of bright yellow, jelly-like material. The serous membranes are shining and smooth.

Lymphatic Glands.—The lumbar chain of glands are enlarged, some of them being the size of a small walnut. These enlarged glands on being cut into are found to be very œdematous, but not hæmorrhagic. The prescapular glands are much enlarged, being 8 cm. (3½ inches) in length.

Circulatory System.—Heart.—The fat of the auriculo-ventricular groove is infiltrated with gelatinous material, which is bright yellow in colour. There are no petechiæ under the epicardium. On opening into the left ventricle many minute petechiæ are seen under the endocardium. The colour of the aorta is bright yellow. The substance of the heart is pale in colour and flabby to the touch. Weighs 125 grammes (4½ ounces).

Respiratory System.—Left lung is purplish in colour, with a dark purple patch in the anterior lobe about the size of half-a-crown. This, on being cut into, shows hæmorrhagic infarction. On section the left lung appears to be fairly healthy. Weighs 210 grammes (7½ ounces). *Right lung.*—Anterior lobe and part of the middle lobe are purplish-red in colour and solid. The anterior lobe sinks in water. The surface is purplish-red in colour, and across the surface is a network of yellow-coloured, clear, jelly-like strands, resembling the lung in a case of horse-sickness. The strands in some places are ¼ inch wide. On section the substance is seen to be hepatised and dark purple in colour, and across the cut section the same network of yellow, gelatinous-like material is seen. The posterior lobe is pale in colour, and on section appears fairly normal. Weighs 345 grammes (12¼ ounces).

Alimentary System.—Spleen is enlarged. 29 cm. in length, 9 cm. broad, and 2·5 cm. in thickness (11″ × 3½″ × 1″). Capsule is purplish in colour. On section the tissue is dark purple in colour and friable. Weighs 245 grammes (8⅝ ounces). *Liver* is bright yellow in colour, tinged with red, like bronze. Capsule is smooth. On section the substance is pale, with congested areas. *Gall-bladder* is distended with thick, greenish-yellow bile. Weighs 890 grammes (31½ ounces).

Fourth Stomach.—Is pale in colour. No ulceration. Intestines not examined.

Urinary System.—Left kidney.—Capsule strips readily. On section the cortical part is seen to be pale, with dilated vessels. Weighs 102 grammes (3½ ounces). *Right kidney*, in a similar condition to the left. Weighs 95 grammes (3¼ ounces).

Experiment 1634.—To study Amakebe in the Calf.

Sept. 4, 1909.—This calf was brought to Mpumu from Kome, one of the Sesse Islands. and was therefore susceptible to Amakebe.
„ 14.—Sent into Kampala, in order to become infected.
„ 24.—Returned from Kampala.
Oct. 4.—Lymphatic glands much enlarged. Oct. 18.—Died.

The following chart represents the course of the disease :—

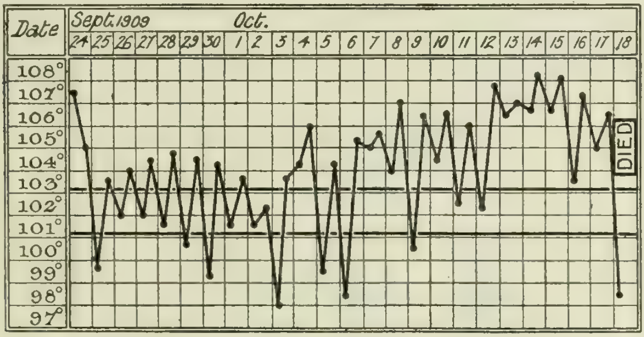

CHART 2.—Experiment 1634. Temperature Curve in a severe and fatal Case of Amakebe.

Oct. 18, 1909.—*Post-mortem* immediately after death.

External Appearances.—Animal about one year old. Preauricular, prescapular, and precrural glands are much enlarged. The prescapular glands measure 3 inches in length and 1½ inches in breadth. On section the glandular tissue is œdematous and, in some places, hæmorrhagic.

Internal Appearances.—On opening into the peritoneal cavity about a gallon of clear, amber-coloured fluid is found. There is a large quantity of yellow, gelatinous infiltration into the omentum. The serous membrane of the omentum is markedly hæmorrhagic, being covered with small petechiæ. The small intestine is dark crimson in colour and intensely congested. The whole of the peritoneal aspect of the diaphragm is covered with small hæmorrhages. On removing the sternum a quantity of yellow, gelatinous material is found in the mediastinum. About 2 ounces of the same clear, amber-coloured fluid are seen in the pleural cavity. The pericardium contains a small quantity of clear, straw-coloured serum.

Circulatory System.—Heart.—A quantity of yellow, gelatinous material is seen at the base. Many small petechiæ both inside and outside the heart.

Respiratory System.—A quantity of white frothy fluid exuded from the nose during the last hours of life. On opening into the *trachea*, however, it is now found to be empty. The *left lung* is partially collapsed and is dark purple in colour. On section the organ is dark crimson in colour and intensely congested. It is, in places, solid in consistence and a portion placed in water sinks. On pressure a white, frothy fluid exudes. *Right lung* is pale in colour, and there are numerous hæmorrhages into the serous membrane. On section it is found to be congested. No part of the lung sinks in water.

Alimentary System.—Spleen is enlarged. Measures 14 inches in length and 4½ inches in breadth. Numerous petechiæ into the capsule. On section the substance is dark in colour, soft, and friable. Weighs 480 grammes (17 ounces). *Liver* is enlarged. On section is seen to be congested. Gall-bladder is distended with dark, olive-green-coloured bile. Weighs 3 lbs. 10 ozs.

Fourth Stomach.—Is reddened, and there are numerous small ulcers in the serous membrane.

Urinary System.—Right Kidney.—Capsule strips readily. There are numerous petechiæ into the capsule. Surface of the organ is injected. On section the kidney is seen to be congested, with many hæmorrhages into the substance. *Left kidney* is in a similar condition to the right.

Experiment 1636.—To study Amakebe in the Calf.

Sept. 4, 1909.—From Kome. Same history as Experiment 1634. Great enlargement of lymphatic glands. Oct. 12.—Died.

The following chart represents the course of the disease :—

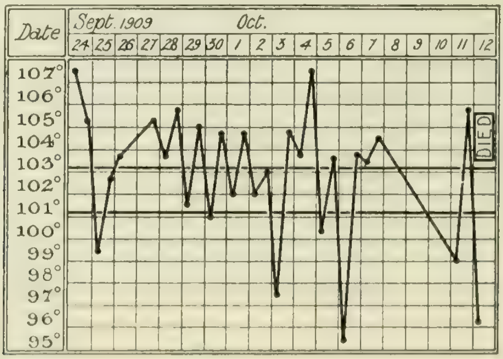

CHART 3.—Experiment 1636. Temperature Curve in a severe and fatal Case of Amakebe.

Oct. 12, 1909.—*Post-mortem* two hours after death.

External Appearances.—This calf has had a running from the nose of clear fluid, which has made a small pool under its head, and at death there was a marked collection of white foam at the nose, like that which occurs in horse-sickness, but not to such an extent.

Internal Appearances.—On removing the skin and opening into the peritoneum, about 4 ounces of clear, straw-coloured fluid is found. The omentum is infiltrated with a yellow jelly-like material. On opening into the thorax, 2 ounces of clear, straw-coloured fluid is seen in the pericardium. About 4 ounces of the same straw-coloured fluid in both pleural cavities. On removing the tongue and trachea a large quantity of jelly-like material is found under the trachea.

Lymphatic Glands.—The prescapular glands are enlarged. One measures 4½ inches in length and 1 inch in thickness. On section it is seen to be dark crimson in colour and hæmorrhagic. The glands generally are enlarged and hæmorrhagic. Some of them show signs of breaking down into pus.

Circulatory System.—*Heart.*—A large quantity of yellow, jelly-like material at the base is seen. There are numerous small petechiæ into the epicardium. None in the endocardium. Muscular substance is pale. Weighs 1 lb. 5 ozs.

Respiratory System.—*Tongue* normal. The *trachea* is full of white froth. Glands at the bifurcation of the trachea are much enlarged and hæmorrhagic. *Right lung*, anterior lobe is dark purple in colour and is found to be the seat of a large infarct. Posterior lobe is also the seat of an infarct at the margin, about 3 inches by 2 inches in extent. On section the substance of the lung is extremely œdematous. A large amount of frothy fluid exudes on pressure. Weighs 2 lbs. 10 ozs. *Left lung* is also the seat of numerous infarcts. On section a large amount of frothy fluid exudes on pressure. Weighs 1 lb. 6 ozs.

Alimentary System.—*Spleen* measures 13 inches in length and 4 inches in breadth. On section the substance is soft and friable. Weighs 13 ounces. *Liver* is enlarged. It is full of flukes. The gall-bladder is distended with dark, chocolate-coloured bile, which contains many flukes. Weighs 8 lbs. 5 ozs.

Fourth Stomach.—The mucous membrane of the fourth stomach is congested. It is dark crimson in colour, and numerous small ulcers are seen scattered throughout.

Urinary System.—*Right Kidney.*—Capsule strips readily. Under the capsule numerous infarcts are seen, about the size of a pea. On section the substance of the organ is congested. Weighs 8 ounces. *Left Kidney.*—Capsule strips readily. In the pelvis of the organ there is a quantity of yellow, jelly-like material. Under the capsule there are also several small infarcts. One of these is as large as a small marble. On section the substance of the kidney is seen to be congested. Weighs 5 ozs.

Experiment 1386.—To study Amakebe in the Calf.

July 26, 1909.—Received from Sir Apolo Kagwa. Had also been kraaled at Kampala for some days.

Aug. 27.—The course of the disease was much the same as in Experiment 1387. The prescapular and other glands became much enlarged, one of them measuring 4 inches in length. By this date the calf had recovered, and was returned to owner.

The following chart represents the course of the temperature :—

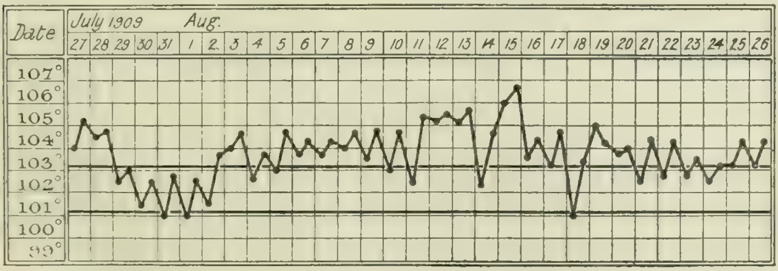

Chart 4.—Experiment 1386. Temperature Curve in a Case of Amakebe ending in Recovery.

Experiment 1635.—To study Amakebe in the Calf.

The following chart represents the course of the temperature :—

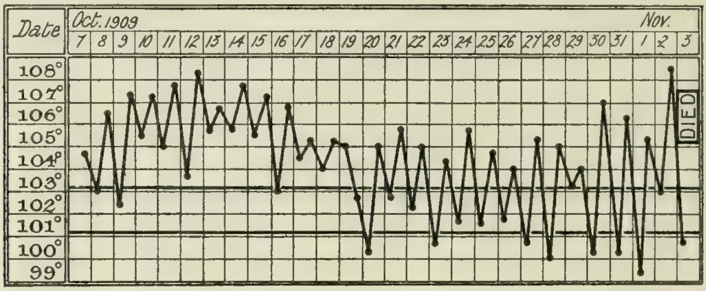

Chart 5.—Experiment 1635. Temperature Curve in a Case of Amakebe ending Fatally.

From these foregoing cases and the *post-mortem* examinations, it will be seen that amakebe is an acute disease of calves, and that the main features of the *post-mortem* are signs of intense anæmia, petechiæ of serous membranes, infiltration of jelly-like material into omentum, anterior mediastinum, base of heart, etc., œdema of lungs, swelling and softening of spleen, hæmorrhagic infarcts into lungs, spleen and kidneys, and sometimes ulceration of the mucous membrane of the stomach.

Piroplasms usually found in the Blood of Uganda Cattle.

When the blood of cattle in Uganda is examined microscopically, two parasites are always to be found, though usually in very small numbers. One of these can readily be recognised as *Piroplasma bigeminum* from its large size and the characteristic appearance of the two pear-shaped bodies (Plate 10, fig. 1). It may, however, also appear as irregularly-shaped, amœboid forms, especially in the spleen (Plate 10, fig. 1). The other parasite is much smaller in size, and is usually seen in the form of a small rod or ring (Plate 10, fig. 2). Both these parasites are inoculable, and appear in the blood of calves without giving rise to any marked disturbance.

Have either of them any connection with Amakebe?

The following experiments go to show that they have not :—

Experiment 556.

To ascertain the effect on a Susceptible Calf of the Injection of Blood containing the Small Rod and Ring-shaped Piroplasm. Will it give rise to Amakebe?

Feb. 22, 1909.—This calf was born last night. To-day the mother was cleared of ticks by hand-picking, and then completely smeared with a mixture of paraffin and cyllin, and mother and calf then placed in a tick-free enclosure.

" 26.—Injected this calf with 5 c.c. blood from calf, Experiment 430, whose blood contains the small rod and ring-shaped piroplasm.

The following chart gives the result :—

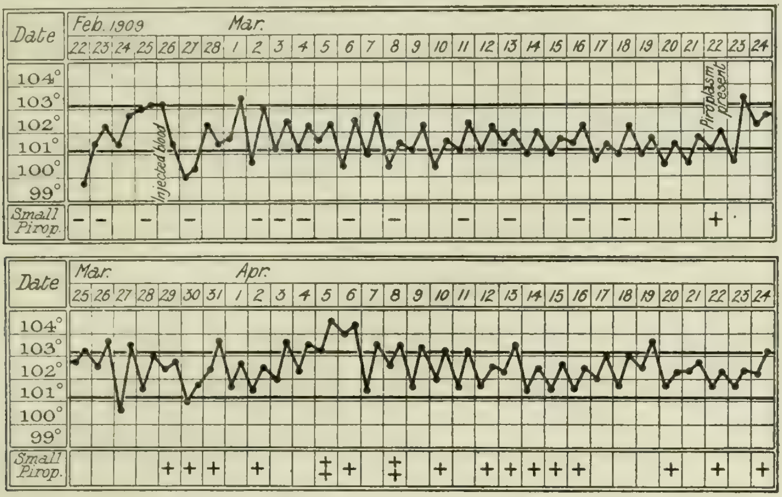

CHART 6.—Experiment 556 represents the Temperature Curve of a Calf which has been Injected with Blood containing the Small Rod and Ring-shaped Piroplasm. The *minus* and *plus* signs show the absence or presence of the small rod and ring-shaped piroplasm in the blood.

Remarks.—Twenty-four days after the injection of the blood containing the small piroplasm, the same rod and ring forms appeared in the blood. The temperature curve hardly shows any response to the invasion of the parasite, and the calf shows no signs of illness. It is evident, then, that the injection of blood containing this small piroplasm gives rise to no symptoms like those seen in Amakebe.

In the same way the injection of blood containing *Piroplasma bigeminum* is followed, after some days, by the appearance of this parasite.

Experiment 1901.

To ascertain if the Injection of Blood containing *Piroplasma bigeminum* will give rise to Symptoms of Amakebe.

Aug. 20, 1909.—Injected 2 c.c. blood containing *Piroplasma bigeminum* into this calf.

The following chart gives the temperature curve :—

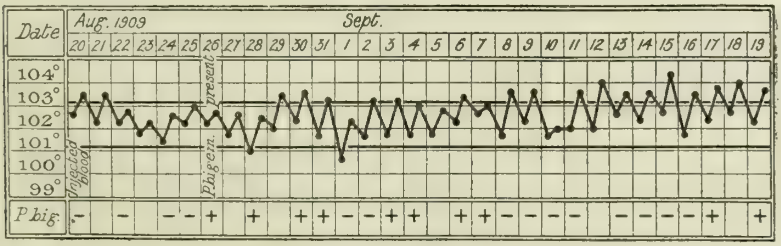

CHART 7.—Experiment 1901 represents the Temperature Curve of a Calf which has been Injected with Blood containing *Piroplasma bigeminum.* The *plus* and *minus* signs show the presence or absence of *Piroplasma bigeminum* in the blood.

Remarks.—Six days after the injection of blood containing the *Piroplasma bigeminum*, this parasite appeared in the blood. The temperature curve is not affected, nor does the calf appear ill. It may, therefore, be concluded that Amakebe is not caused by the injection of blood containing either *Piroplasma bigeminum* or the small rod and ring form.

It is well known that *Piroplasma bigeminum* is carried from affected to susceptible animals by different varieties of the blue tick, as well as other species of ticks. It would seem that the small rod and ring form of piroplasm is carried by the brown tick, as the following two experiments will show.

Experiment 747.

To ascertain if Brown Nymphs which had fed as larvæ on an Animal whose blood contained the Small Rod and Ring Forms, are capable of carrying them to a Susceptible Animal, and if the disease so set up will have the Symptoms of Amakebe.

June 24, 1909.—This calf, like the others, has been brought up in a tick-free shed. It has been under observation since May 10 without showing any small rod and ring forms in its blood. To-day, a large number of brown nymphs which had fed as larvæ on an ox whose blood contained the small rod and ring piroplasm were placed on this calf.

The following chart shows the course of the temperature :—

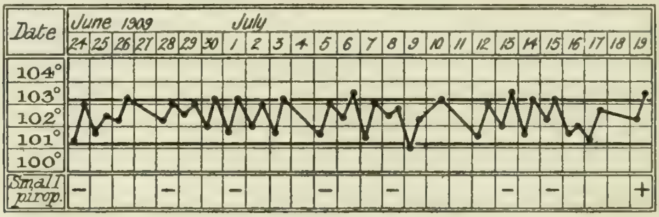

Chart 8.—Experiment 747 represents the Temperature Curve of a Calf upon which Infected Brown Nymphs have been fed. The *minus* and *plus* signs show the absence or presence of the small rod and ring piroplasm in the blood.

Remarks.—Twenty-five days after the infected brown nymphs were fed on this calf the small rod and ring-shaped piroplasm appeared in the blood. The temperature curve is not affected, and the calf shows no signs of Amakebe. It is evident, then, that the small rod and ring-shaped piroplasms transferred to a susceptible calf by means of brown nymphs do not give rise to amakebe.

Experiment 659.

To ascertain if Adult Brown Ticks which had Fed as Nymphs on an Animal whose Blood contained the Small Rod and Ring Forms are capable of carrying them to a Susceptible Animal and setting up the Symptoms of Amakebe.

Aug. 23, 1909.—This calf was born on April 4 in a tick-free shed. It has been examined almost daily since that date, and up to the present has shown no parasites of any kind in its blood. To-day, a large number of adult brown ticks were placed on this calf.

The following chart shows the course of the temperature :—

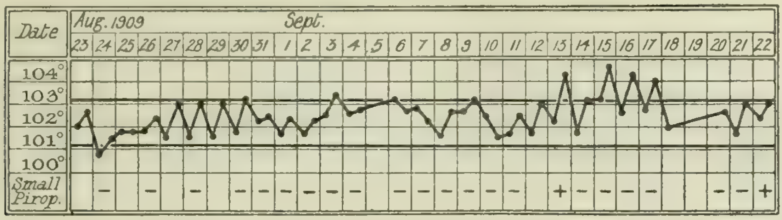

Chart 9.—Experiment 659 represents the Temperature Curve of a Calf upon which Infected Brown Adult Ticks had been fed. The *minus* and *plus* signs show the absence or presence of the small rod and ring piroplasm in the blood.

Remarks.—Twenty-one days after the infected brown adults had fed on this calf the small piroplasm appeared in the blood. The temperature curve is only slightly affected, and the calf shows no symptoms of Amakebe.

From the foregoing experiments it may be concluded, then, that the appearance of *Piroplasma bigeminum* or of the small rod and ring form of piroplasm in the blood of a susceptible calf, whether introduced by the injection of blood or, in the case of the latter, by the agency of the brown tick, is not accompanied by the symptoms of Amakebe. It also is seen from these experiments that the small rod and ring form is inoculable, is carried by the brown tick, and the incubation period is long. This corresponds with the description given by Dr. Theiler, Pretoria, of the piroplasm discovered by him in the Transvaal, and named by him *Piroplasma mutans.*

We may, therefore, consider that the two piroplasms which constantly occur in the blood of Uganda cattle are those known as *Piroplasma bigeminum* and *Piroplasma mutans,* and that neither is the cause of Amakebe.

Is Amakebe Inoculable?

It has been shown that blood containing either *Piroplasma bigeminum* or *Piroplasma mutans* if injected into susceptible cattle will give rise to these diseases. Is it equally true that Amakebe is inoculable? The following experiments were carried out to obtain an answer to this question :—

Experiment 1902.

To ascertain if Blood taken from an Animal suffering from Amakebe and injected into a susceptible Calf will give rise to the Disease.

Feb. 22, 1909.—This calf was born last night. Placed in tick-free shed.
„ 26.—Injected with a 5 c.c. blood from calf, Experiment 430, suffering from Amakebe.

The following chart shows the result :—

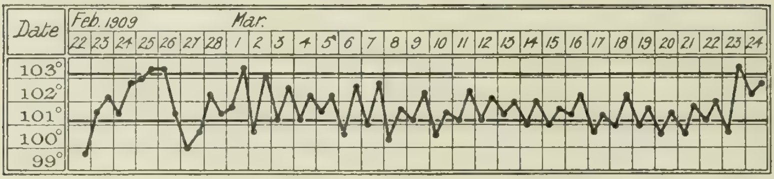

CHART 10.—Experiment 1902, represents the Temperature Curve of a Calf into which Blood from a Case of Amakebe has been injected.

Remarks.—The temperature curve is not disturbed by the injection of Amakebe blood, nor is the calf affected in any way.

Experiment 1903. (The above experiment repeated.)

Aug. 20, 1909.—Injected 5 c.c. mixture of blood and gland-juice from calf, Experiment 1387, which is suffering from Amakebe.

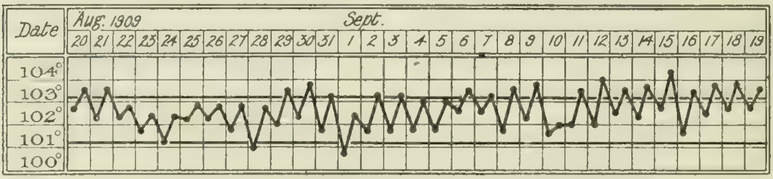

CHART 11.—Experiment 1903, represents the Temperature Curve of a Calf into which Blood from a Case of Amakebe has been injected.

Remarks.—The result of the injection of Amakebe blood is again negative.

Experiment 1904. (The above experiment again repeated.)

Aug. 21, 1909.—Injected Amakebe blood.

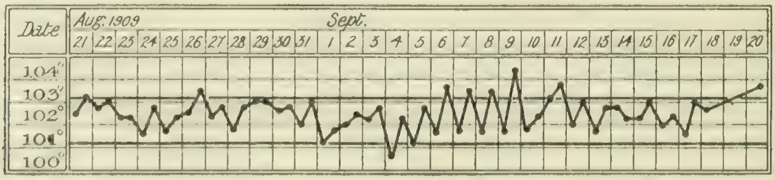

CHART 12.—Experiment 1904, represents the Temperature Curve of a Calf into which Blood from a Case of Amakebe has been injected.

Remarks.—Result negative.

Experiment 1905.

Sept. 24, 1909.—Injected Amakebe blood.

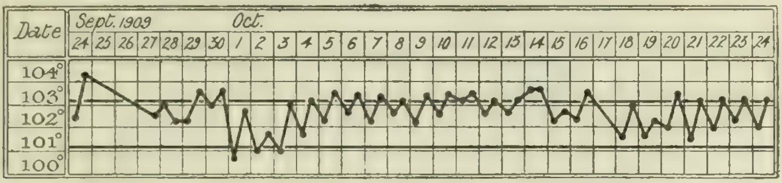

Chart 13.—Experiment 1905, represents the Temperature Curve of a Calf into which Blood from a Case of Amakebe has been injected.

Remarks.—Result negative.

On three other occasions (Experiments 659, 1585 and 1586) was this experiment repeated, and always with a negative result.

It may be concluded, then, that Amakebe differs from *Piroplasma bigeminum* and *Piroplasma mutans*, in that it is not inoculable, whereas the latter diseases are.

Result of Exposing Susceptible Calves in a Kraal contaminated by Amakebe.

Kampala, the native capital of Uganda, has a bad reputation for Amakebe. This is probably due to the number of calves stabled in the vicinity. Kampala has a large population of both Europeans and natives, and the milk supply is obtained from private cows kept in the town. The herds of cattle belonging to different individuals are grazed in various parts of the country, but as soon as a cow has calved, she is sent into Kampala to provide milk for her owner. Almost all the calves brought in die of Amakebe, which brings about an unhealthy state of things in the cattle kraals where the calves are kept during the day.

The following experiments will show the effect of exposing susceptible calves for a few days in a Kampala cattle kraal:—

Experiment 1590.

To ascertain the Effect of exposing a susceptible Calf in a Kraal contaminated by Amakebe.

Oct. 11, 1909.—Sent this calf into Kampala. Oct. 17. -Returned from Kampala.

The following chart shows the course of the temperature, and the presence or absence of *Piroplasma bigeminum* or the small rod-and-ring-formed piroplasma in the blood.

Remarks.—The result of exposing this calf to a contaminated kraal is an attack of Amakebe, characterised by high fever, swollen glands, and death.

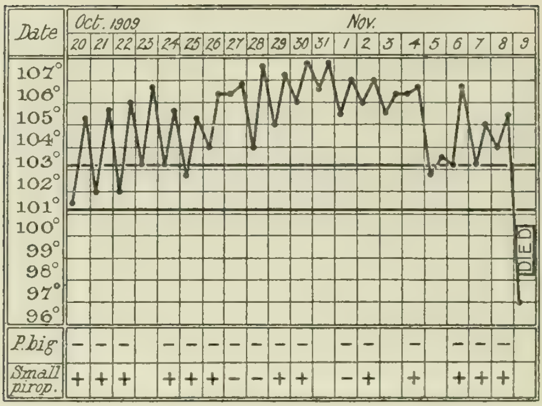

CHART 14.—Experiment 1590, represents the Temperature Curve of a Calf which has been exposed in a Kraal contaminated by Amakebe. The *plus* and *minus* signs show the presence or absence of *Piroplasma bigeminum* or the small rod and ring piroplasm in the blood.

Experiment 1593.

To ascertain the Effect of exposing a susceptible Calf, as in the previous Experiment.

Oct. 11, 1909.—This calf sent into Kampala. Oct. 17.—Returned from Kampala.

The following chart represents the course of the disease, and the presence or absence of *Piroplasma bigeminum* or the small piroplasm in the blood :—

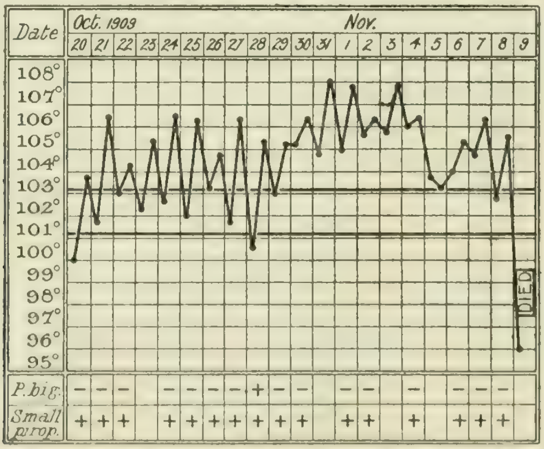

CHART 15.—Experiment 1593, represents the Temperature Curve of a Calf which has been exposed in a Kraal contaminated by Amakebe. The *plus* and *minus* signs show the presence or absence of *Piroplasma bigeminum* and the small rod and ring piroplasm in the blood.

It is evident, then, that the exposure of susceptible calves for a few days in a kraal where Amakebe is common is followed by a serious illness. There is high fever, glandular enlargement, emaciation, and, as a rule, death. This disease has been shown to be caused neither by *Piroplasma bigeminum* nor *Piroplasma mutans.* What, then, is it caused by?

Examination of the Blood in Amakebe.

When the blood of an animal suffering from Amakebe is examined, many small piroplasms will be seen (Plate 10, fig. 3), which appear to be of the same size and shape as *Piroplasma mutans,* and sometimes a few *Piroplasma bigeminum*; otherwise, no new parasite can be said to have come into the blood. This increase in the number of the small piroplasms in the blood of a calf suffering from Amakebe may be explained by saying that the severe illness has led to an excessive multiplication of the *Piroplasma mutans* which was already in the blood. Or, on the other hand, it may be that another species of piroplasm, similar in size and shape to *Piroplasma mutans,* has appeared in the blood, and that the phenomena of Amakebe are due to it.

Marginal Points.—Besides the large and small piroplasms, another kind of body is found in the red blood corpuscles, which Theiler has called *marginal points.* In a lecture delivered by him in August, 1909, at Nairobi, in British East Africa, and published in the 'Agricultural Journal of British East Africa,' October, 1909, he states: "I have recently come to the conclusion that the disease called gall-sickness, and hitherto looked on as a sequel of redwater, is due to the presence of another parasite, which I have called 'Marginal Points' owing to their position in the red blood corpuscles. Gall-sickness is, therefore, a separate and distinct disease." Dr. Theiler considers it proved that this new disease is transmitted by the blue tick. This all shows how complicated and difficult to distinguish are the diseases of cattle. An ox may have *Piroplasma bigeminum,* small rod-and-ring-shaped piroplasms, marginal points, and one or two species of trypanosome in its blood at the same time. To which parasite have the different phenomena of the disease to be credited?

The two following tables give the blood examination in two cases of Amakebe, and illustrate this complexity.

The marginal points are small, deeply-staining bodies, usually placed near the edge of a red blood corpuscle (Plate 10, fig. 3). If these bodies really constitute a new and undescribed parasite, the discovery will be one of the greatest interest. Bodies similar in every way to these are found, however, in healthy young rats, goats, calves, etc., so that it is difficult to believe at once in their parasitic nature. Rather would they appear to be cell enclosures, due to rapid changes taking place in the blood, such as take place in young animals or in anæmias. In Amakebe they are sometimes very numerous, and it requires no great stretch of the imagination to see in them the youngest

Experiment 1387.—Blood Examination in a Case of Amakebe.

Date.	Parasites in blood.			No. of red blood corpuscles in 1 c. mm. of blood. Normal 10,000,000.
	Piroplasma bigeminum.	Small rod and ring forms.	Marginal points.	
1909.				
July 28	—	—	+	
29	—	+	—	
30	—	—	+	
31	—	—	+	
Aug. 1	—	—	—	
2	—	—	+ +	5,420,000
3	—	+	+ +	5,670,000
4	—	+	—	
5	—	—	+	
6	—	+	+ +	5,680,000
7	—	+	+ +	
8	—	+	+	
9	—	+	+	
10	—	—	+	
11	—	—	+ +	4,420,000
13	—	+	+ + +	
14	—	+	+ + +	
16	—	—	+ + +	4,540,000
19	—	+	+ + +	4,500,000
23				2,890,000
24	+ + +	+	+	2,820,000

Table I, Experiment 1387. The parasites to be found in a case of Amakebe. The *plus* and *minus* signs show the presence or absence of these bodies in the blood. The fourth column gives the number of red blood corpuscles in a cubic millimetre. + present, + + numerous, + + + very numerous.

Experiment 1636.—Blood Examination in a Case of Amakebe.

Date.	Parasites in blood.			
	Piroplasma bigeminum.	Small rod and ring forms.	Marginal points.	*Trypanosoma vivax.*
1909.				
Sept. 24	—	—	—	—
27	+	+	+	—
28	—	+	—	—
29	—	—	—	—
30	—	—	—	—
Oct. 1	—	—	—	+
2	+	+	—	—
4	—	—	—	—
5	—	+	—	—
6	—	+ +	—	—
7	—	+	+	—
8	—	+	—	+
9	—	+	+	+
11	+	+	+	+
12	+	+	+	+

Table II, Experiment 1636. Parasites found in a case of Amakebe. The *plus* and *minus* signs show the presence or absence of these bodies in the blood.

Remarks.—From these two tables it will be seen that small piroplasms and marginal points are commonly found in Amakebe, and that trypanosomes may also be present.

stage of the intra-corpuscular parasite, which from being round becomes wedge-shaped, oval or circular and rod-shaped. It may be that both these views are true—that some of the so-called marginal points are remains of chromatin from some previous nuclear structure, and that others are the earliest stages of an intra-corpuscular parasite. More work is required before any definite conclusion can be arrived at.

Koch's Granules or Blue Bodies.—Another body which may sometimes, though rarely, be seen in the blood of Amakebe calves, is one similar to that first described by Koch, and known as Koch's Granules or Blue Bodies. They are found principally in the spleen, lymphatic glands and liver, where they may be quite numerous. Stained by Giemsa the body appears as a blue-coloured cell, filled with coarse chromatin granules (Plate 10, fig. 5).

The following table gives cases of Amakebe in which these bodies were found :—

Experiment.	Date.	Spleen.	Liver.	Lymphatic glands.	Kidney.	Lung.	Blood.
	1909.						
415	May 10	+					
1392	July 24	+					
1593	Nov. 10	+					
1633	Oct. 5	+ + +	+ + +	+ + +			
1634	18	+ +	+ +	+	+	+	
1635	15						+
1636	12	+	+	+	+	+	
1637	6	+	+	+	+	+	
1638	6	+ +	+	+ +	+	+	+
1833	—	+	+		+		
1888	Nov. 5	+ +					
1891	8	+					
1908	14	+ +					

Table III, showing the presence of blue bodies in cases of Amakebe. + present, + + numerous, + + + very numerous, — absent.

Diagnosis of Amakebe.

What, then, is Amakebe? In the opinion of the Commission it is the disease of cattle discovered by Koch, and named by him East Coast Fever. The chief grounds for this opinion are, the symptoms during life, the appearances after death, the occurrence of a small piroplasm in the blood indistinguishable from *Piroplasma parvum*, and lastly and chiefly, the presence of the blue bodies in the spleen and other organs. These bodies have never been known to occur in any other disease, and the diagnosis of East Coast Fever is made in South Africa if such bodies are found in spleen smears.

Conclusions.

1. The blood of cattle in Uganda almost always contains *Piroplasma bigeminum* and *Piroplasma mutans*, and the cattle are therefore immune to these two diseases.

2. The disease of calves called Amakebe is East Coast Fever, so that very many of the cattle in Uganda are almost immune to this disease.

3. Owing to the nature of East Coast Fever, inasmuch as animals recovered from the disease are no longer infective, some calves may escape attack of Amakebe, and so remain susceptible.

4. Thus the calves of the Sesse Islands escape Amakebe, and when as grown-up cattle they are transferred to the mainland, they mostly die of East Coast Fever.

5. The carriers of East Coast Fever—*Rhipicephalus appendiculatus*, or brown tick; *Rhipicephalus evertsi*, or red-legged tick; and *Rhipicephalus simus*—are all common in Uganda.

DESCRIPTION OF PLATE.

Fig. 1.—The two upper corpuscles show the characteristic pear-shaped forms of *Piroplasma bigeminum* as they appear in the blood. The lower amœboid forms are drawn from a preparation of spleen. Stained Giemsa. × 2000.

Fig. 2.—*Piroplasma mutans* in the blood. Stained Giemsa. × 2000.

Fig. 3.—The small rod-and-ring-shaped piroplasm, as seen in the blood of a case of Amakebe. Among them are the deeply-stained bodies known as marginal points. Stained Leishman. × 2000.

Fig. 4.—Red blood corpuscles containing piroplasms from the spleen of a case of Amakebe. Stained Giemsa. × 2000.

Fig. 5.—Koch's granules or blue bodies from the spleen of a case of Amakebe. Stained Giemsa. × 2000.

The Transmission of Trypanosoma lewisi *by the Rat-flea* (Ceratophyllus fasciatus). (*Preliminary Communication.*)

By E. A. MINCHIN, M.A., and J. D. THOMSON, M.A., M.B., C.M.

(Communicated by Sir Ray Lankester, K.C.B., F.R.S. Received January 7,—Read February 3, 1910.)

Since the spring of 1909 we have been engaged in conducting experiments and observations upon the transmission of *Trypanosoma lewisi* by rat-fleas, and we hope at some future period to publish a full account of the whole work, including the development of the trypanosome in the flea. The experiments here recorded have been selected for special publication because they are complete in themselves and because they appear to us decisive on a point of fundamental importance, namely the method of transmission by the flea.

Nuttall (1908) reviews the subject of the transmission of *T. lewisi* and records experiments of his own on that subject up to December, 1908. Reference will be made to Nuttall's experiments later on, and we will first proceed to describe the experiments that form the subject of the present communication under the following headings:—

I. The object of the experiments.
II. Account of the experiments (general and detailed).
III. Conclusions to be drawn from the results of the experiments.

I. *The Object of the Experiments* is chiefly to make clear the *method* of transmission; subdivided it may be stated thus:—first and incidentally, to confirm conclusions that fleas are capable of transferring *T. lewisi* from infected to "clean" (*i.e.* non-infected) healthy rats, but secondly and principally, to determine the mode of infection, whether "direct" or "cyclical," and thirdly, when the cyclical method was indicated by the progress of the experiments, to confirm results and to ascertain further facts connected with the cyclical method.

In order to avoid confusion and misconception, it may be well to make clear what we mean by the terms "direct" and "cyclical" in this connection. The Editor of the Bulletin issued from the Sleeping Sickness Bureau (1909) writes:—"There are two methods of transmission of trypanosomes generally recognised: (*a*) that which is mechanical, dependent on physical conditions alone, and (*b*) that which occurs after a 'cycle of development,' an expression which implies the conjugation of two individuals. The work of Novy and MacNeal has shown that a third method (*c*) is possible, partly mechanical and partly biological, resulting from the late multiplication of the parasites in the intestine and their subsequent introduction into the body of the bitten animal." It may be pointed out, first that the mechanical element is present equally in (*a*), (*b*), and (*c*), and secondly that the question of "the conjugation of two individuals" seems to us of quite secondary importance in this connection. There are non-sexual cycles of development as well as sexual; in the malarial parasite, for instance, there is a non-sexual cycle, which takes place in the human body, and a sexual cycle, which takes place in the mosquito. At the present time the belief that a sexual cycle in the development of trypanosomes takes place in the invertebrate host is largely an assumption, based on the analogy of the malarial parasite, and in need of objective proof.

We desire to approach the subject in an unbiased manner, and we would recognise, for the present at least, only two methods of transmission, of fundamental scientific and practical importance, which we shall term the "direct" and "cyclical" methods respectively. In the direct method the invertebrate acts merely as a suitable instrument in the transmission. Experimentally the chances that the invertebrate will convey the infection to a susceptible animal by the direct method are greatest immediately after it has contaminated its proboscis by feeding on an infected one, and these chances gradually diminish and cease altogether within a comparatively limited period of time. And experimentally the invertebrate is found to have exhausted its power to infect in the process of cleaning its proboscis at the feed which follows next after that on the infected animal. In the cyclical method, on the other hand, the invertebrate is more than a suitable instrument in the transmission: it acts as a host in which the parasite establishes itself and maintains the existence of its species. Infection by the cyclical method can take place only after the parasite has established itself, and it can then continue to take place so long as the parasite maintains its existence in the invertebrate. Experimentally it is found that an invertebrate of the right species, after having fed on an infected animal, may feed many times on susceptible animals without conveying the infection, and may then, without having fed again on an infected animal, become infective; and, further, that once infective, it may remain infective for an indefinite period of time—possibly for the rest of its life. Of such an invertebrate we may say that it is a true host, and that the parasite it transmits passes through a cycle of development within it, meaning by the word cycle a series of changes and generations which follow one another in more or less definite order in the development and multiplication of the parasite, and leaving it an open question whether or not a sexual process takes place in the course of the development.

Experiments A and B were arranged so as to eliminate possible infection other than by fleas, and to separate "direct" from "cyclical" infection, so that if infections did take place, it would be clear whether they were the result of the direct method, the cyclical method, or both. When it had been ascertained in the course of Experiments A and B that infections did take place, but not by the direct method, these experiments were prolonged in order to determine if fleas, once infective, retain the infection so as to infect a succession of healthy, clean rats. This is also a clear issue in Experiments C and D, which were arranged with a view to determine further by direct observation, and within narrow limits, (1) the length of the incubation-period in the flea, that is to say, the length of time required for the parasite to establish itself in the flea and render it infective, and (2) the length of the multiplication-period in the rat, that is to say, the length of time from the actual inoculation of the parasite into the rat until the trypanosomes cease to multiply in the rat's blood. As is well known, when a rat is first infected with *T. lewisi*, the parasite multiplies in the blood very rapidly. After a certain length of time the multiplication ceases entirely and rather suddenly. When rats are infected artificially in the laboratory by the ordinary method of intra-peritoneal inoculation of blood from an infected rat, the multiplication-period lasts for some 10 or 12 days after inoculation, as a rule; but it by no means follows that multiplication lasts for the same length of time when the inoculation is performed by fleas. If the length of the multiplication-period is known, and is found to be a constant, it is evident that in any given experiment the time when the infection of the rat took place can be ascertained by simply observing when multiplication of the trypanosome ceases in the blood and then deducting the known period; and further, if the infection is a first one and the time during which the fleas were exposed to infection was short and is known, then the length of the incubation-period in the flea can be calculated also. Experiments D and E were undertaken with the special object of determining the incubation-period in the flea.

II. *Account of the Experiments.*

(1) *General.*—The same arrangements were made for all the experiments, which differ only in details. The fleas used in all cases had been bred in flea-proof cages in the laboratory, the parents being some 50 fleas which were obtained in the early autumn of 1908 from rats trapped in the neighbourhood of the Sutton Broad Laboratory in Norfolk. These fleas multiplied in our cages during last winter and spring to produce literally thousands; we have

no difficulty in obtaining 200 fleas from the breeding cages for an experiment when required. The fleas are kept alive in the cages by being fed on non-infected healthy tame rats.

Although it is not known whether any of the rats on which the original 50 fleas were caught were infected with *T. lewisi* or not, it is quite certain that the stock of fleas in our breeding cages is free from infection, since not one of the many non-infected rats used for feeding them has ever become infected. We are indebted to the Hon. N. C. Rothschild for kindly identifying the genus and species of our fleas as *Ceratophyllus fasciatus*.

In preparing for each of these experiments, the first step was to collect a sufficient number of fleas, usually about 200, from the breeding cages. These fleas were then exposed to infection by being put, together with a heavily-infected rat, into a tin cage specially constructed to facilitate the recovery of the fleas. The infected rat itself was ascertained to be free from fleas before it was put into the tin cage; and the fleas from the breeding cages were left with it in this cage for a known period of time. At the end of this period as many as could be recovered from the infected rat and from the tin cage were used to colonise a freshly prepared cage, constructed on the model used by the Indian Plague Commission for their experiments on rats and fleas.* This cage, which we will call Cage X, was carefully cleaned and disinfected before being used for the experiment, and into it a healthy, non-infected rat (Rat X 1) was now introduced, together with the fleas collected from the tin cage already mentioned. Three or four days later, or when under the special conditions a sufficient time had elapsed to ensure that any flea that survived must have fed on Rat X 1, this rat was removed from Cage X, and was carefully cleaned from fleas by the aid of chloroform-vapour, after which it was put into a fresh cage by itself. The fleas removed from Rat X 1, after they had recovered from the chloroform-vapour, were put back into Cage X. Another healthy, non-infected rat (Rat X 2) was then put into Cage X, and so on with Rat X 3, X 4, etc. (each treated similarly to Rat X 1). Each of these rats, after removal from Cage X, was then examined daily or every other day subsequently for trypanosomes in its blood.

(2) *Detailed Account.*

Experiment A.

4.10.09.—200 fleas taken from the breeding cage were put into the special tin cage together with a heavily-infected tame rat showing many trypanosomes in its blood.

8.10.09.—44 fleas recovered from the above-mentioned cage were put into a freshly-prepared flea-proof cage (Cage A), together with a clean, healthy rat (Rat A 1).

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* See 'Journal of Hygiene,' vol. 6, 1906, p. 435, pl. 4.

6 other fleas recovered from the tin cage were dissected ; in 5 of them trypanosomes were found, and in one instance multiplying forms were seen in the rectum. (The recta of the others were not specially examined.)

12/10/09.—Rat A 1 was removed from Cage A, was carefully cleaned from fleas by the aid of chloroform-vapour, and was put into a separate cage by itself ; two fleas removed from it were dissected. A healthy, clean tame rat (Rat A 2) was then put into Cage A.

Rat A 1 was examined regularly from 16/10/09 to 13/11/09, but no trypanosomes were found. On 15/11/09 Rat A 1 was found dead ; no trypanosomes were found in its blood.

On 26/10/09 Rat A 2 was found dead ; no trypanosomes were found in its blood.

27/10/09.—A healthy, non-infected tame rat (Rat A 3) was put into Cage A, whence the dead Rat A 2 had been removed the day before.

6/11/09.—Rat A 3 examined, no trypanosomes found.
8/11/09.— „ „ „ „
10/11/09.— „ „ „ „

11/11/09.—Rat A 3 examined, trypanosomes found ; a smear made showed multiplying forms.

13/11/09.—A smear made from the blood of Rat A 3 showed trypanosomes abundant, still multiplying forms.

15/11/09.—A smear of the blood of Rat A 3 showed very few multiplying forms.

16/11/09.—A smear from Rat A 3 showed the trypanosomes all of the adult form ; multiplication ended.

Tabular Summary of Experiment A.

Cage A colonised with 44 fleas that had been exposed to infection from October 4 till October 8.

Rat.	Put in.	Taken out.	Result.	Trypanosomes first seen.	Multiplication ended.
A 1	October 8	October 12	0	—	—
A 2	„ 12	Found dead, Cage A, on October 26; no trypanosomes seen in its blood.			
A 3	„ 27	Left in	+	November 11	November 16

Experiment B.

11/10/09.—200 fleas taken from the breeding cage were put into the special tin cage together with a heavily-infected tame rat showing many trypanosomes in its blood (the same rat that was used in Experiment A).

15/10/09.—157 fleas recovered from the above cage were put into a freshly-prepared, flea-proof cage (Cage B), together with a healthy, non-infected tame rat (Rat B 1). Two fleas were dissected, multiplying forms of trypanosomes were found in the rectum of one.

19/10/09.—Rat B 1 was removed from Cage B, was carefully freed from fleas by the aid of chloroform-vapour, and was put into a separate cage by itself. Of 26 fleas removed from Rat B 1, 21 were put back into Cage B, and the other 5 were dissected ; in one of the fleas dissected multiplying forms of trypanosomes were found in the rectum, and in another multiplying forms were found in the rectum and in one of the Malpighian tubes. A healthy, non-infected tame rat (B 2) was put into Cage B.

Rat B 1 was examined regularly from 23/10/09 till 8/11/09; no trypanosomes were found at any time in its blood.

8/11/09.—Rat B 2 examined, many trypanosomes found in its blood; all adult forms, apparently just past the multiplication-period.

Tabular Summary of Experiment B.

Cage B colonised with 157 fleas that had been exposed to infection from October 11 till October 15.

Rat.	Put in.	Taken out.	Result.
B 1	October 15	October 19	0
B 2	„ 19	Left in	+

Having confirmed conclusions that fleas are capable of transferring *T. lewisi* from infected to clean, healthy rats, and having shown that infection did not take place by the direct method, Experiments A and B were prolonged in order to ascertain whether fleas once infective retain infection so as to be capable of infecting a series of clean, healthy rats. In both cases it was found that fleas once infective do so retain the infection, and can infect a series of rats without themselves being exposed to fresh infection, but as this point is so clearly brought out in Experiment C which follows, the detailed accounts of the prolongations of Experiments A and B have not been added.

Experiment C.

24/11/09.—206 fleas taken from the breeding cage were put into the special tin cage together with a heavily-infected tame rat, showing many trypanosomes in its blood. This rat had been submitted to chloroform-vapour immediately before and carefully searched to ensure that it harboured no fleas.

27/11/09.—160 fleas recovered from the above cage were put into a freshly prepared, flea-proof cage (Cage C), together with a healthy, clean tame rat (Rat C 1). 5 fleas were dissected, and in all trypanosomes were found. In the rectum of 1 flea (♀) large masses of multiplying Crithidia-like forms were found. In the rectum of another flea (♂) multiplying clumps were also found.

30/11/09.—Rat C 1 was removed from Cage C, was carefully freed from fleas by the aid of chloroform vapour, and was put into a separate flea-proof cage by itself. A healthy, clean tame rat (C 2) was put into Cage C.

Rat C 1 was examined regularly from 1/12/09 until 3/1/10. No trypanosomes were found in its blood at any time.

3/12/09.—Rat C 2 was removed from Cage C, was carefully freed from fleas, and was then put into a separate freshly-prepared flea-proof cage by itself. 11 fleas recovered from it were put back into Cage C. A healthy, clean tame rat (Rat C 3) was then put into Cage C.

Rat C 2 was examined daily from 4/12/09, and on 10/12/09 trypanosomes were first seen in its blood. Smears were made daily of the blood until 15/12/09, at which date the multiplication-period came to an end.

6/12/09.—Rat C 3 was removed from Cage C, was carefully freed from fleas, and was put into a fresh cage by itself. 2 fleas recovered from it were put back into Cage C. A healthy, clean tame rat (C 4) was then put into Cage C.

Rat C 3 was examined daily from 6/12/09 until 11/12/09, and again on 13/12/09, when trypanosomes were first seen in its blood. Permanent smears of its blood were made daily from 13/12/09 till 18/12/09, when the multiplication-period ended.

8/12/09.—Rat C 4 was removed from Cage C, was carefully freed from fleas, and was then put into a separate cage by itself. 3 fleas recovered from it were put back into Cage C. A healthy, clean tame rat (Rat C 5) was then put into Cage C.

Rat C 4 was examined daily from 8/12/09 to 11/12/09, and again on 13/12/09, when trypanosomes were first found in its blood. Permanent smears were made daily of its blood from 13/12/09 till 18/12/09, when the multiplication-period had practically ended.

10/12/09.—Rat C 5 was removed from Cage C, was carefully freed from fleas, and was then put into a fresh cage by itself. 14 fleas recovered were put back into Cage C. A healthy, clean tame rat (Rat C 6) was then put into Cage C.

Rat C 5 was examined regularly from 11/12/09 till 14/12/09, when trypanosomes were first seen in the blood. Permanent smears were made of its blood from 14/12/09 till 21/12/09, when the multiplication-period came to an end.

11/12/09.—Rat C 6 was removed from Cage C, was carefully freed from fleas, and was then put into a separate cage by itself. 20 fleas recovered from it were put back into Cage C. A healthy, clean tame rat (Rat C 7) was then put into Cage C.

Rat C 6 was examined regularly from 13/12/09 till 16/12/09, when trypanosomes were first found in its blood. Permanent smears of its blood were made from 16/12/09 till 23/12/09, when the multiplication-period came to an end.

13/12/09.—Rat C 7 was removed from Cage C, was carefully freed from fleas, and was put into a separate cage by itself. 16 fleas recovered from it were returned to Cage C. A healthy, clean tame rat (Rat C 8) was then put into Cage C.

Rat C 7 was examined regularly from 15/12/09 till 17/12/09, when trypanosomes were first seen in its blood. Permanent smears were made of its blood from 17/12/09 till 24/12/09, when the multiplication-period came to an end.

15/12/09.—Rat C 8 was removed from Cage C, was carefully freed from fleas, and was put into a separate cage by itself. 5 fleas recovered from it were returned to Cage C. A healthy, clean tame rat (Rat C 9) was then put into Cage C.

Rat C 8 was examined regularly from 16/12/09 till 20/12/09, when trypanosomes were first seen in its blood. Permanent smears were made from 20/12/09 till 26/12/09, when the multiplication-period came to an end.

17/12/09.—Rat C 9 was removed from Cage C, was carefully freed from fleas, and was put into a separate cage by itself. 23 fleas recovered from it were put back into Cage C. A healthy, clean tame rat (Rat C 10) was then put into Cage C.

Rat C 9 was examined regularly from 18/12/09 till 21/12/09, when trypanosomes were first seen in its blood. Permanent smears were made from 21/12/09 till it was found dead on 28/12/09. On this date the multiplication-period had not quite ended, but from appearances would probably have ended on the following day, 29/12/09

20/12 09.—Rat C 10 was removed from Cage C, was carefully freed from fleas, and was then put into a separate cage by itself. 6 fleas recovered from it were put back into Cage C. A healthy, clean rat (Rat C 11) was then put into Cage C.

Rat C 10 was examined regularly from 21/12/09 till 26/12/09, when trypanosomes were first seen in its blood. Permanent smears were then made from 26/12/09 till 30/12/09, when the multiplication-period came to an end.

22/12/09.—Rat C 11 was removed from Cage C, was carefully examined for fleas by aid of chloroform-vapour, but no flea was found on it. It was put into a separate cage by itself. A healthy, clean tame rat (Rat C 12) was then put into Cage C.

Rat C 11 was examined regularly from 23/12/09 till 28/12/09, when trypanosomes were first seen in its blood. Permanent smears were then made from 28/12/09 till 1/1/10, when the multiplication-period came to an end.

24/12/09.—Rat C 12 was removed from Cage C, was carefully freed from fleas, and was put into a separate cage by itself; 9 fleas recovered from it were returned to Cage C. A healthy, clean tame rat (Rat C 13) was then put into Cage C.

Rat C 12 was examined on 25/12/09 and was found dead on 26/12/09 before trypanosomes had time to appear in its blood.

28/12/09.—Rat C 13 was removed from Cage C, was carefully freed from fleas, and was put into a separate cage by itself; 3 fleas recovered from it were returned to Cage C. A healthy, clean tame rat (Rat C 14) was then put into Cage C.

Rat C 13 was examined regularly from 29/12/09 till 3/1/10, when trypanosomes were first seen in its blood. Permanent smears were made from 3/1/10 till 8/1/10, when the multiplication-period came to an end.

31/12/09.—Rat C 14 was removed from Cage C, was carefully searched, but no flea found on it, and was put into a separate cage by itself. A healthy, clean tame rat (Rat C 15) was then put into Cage C.

Rat C 14 was examined regularly from 1/1/10 till 22/1/10. No trypanosomes were found in its blood at any time.

(Experiment still proceeding—see below.)

Experiment D.

6/12/09.—254 fleas from the breeding cage were put into the freshly-prepared and cleaned special tin cage.

7/12/09.—A heavily-infected tame rat showing many trypanosomes in its blood, after being carefully searched with chloroform-vapour to ensure that it harboured no fleas, was put into the above-mentioned tin cage at 2 p.m.

8/12/09.—212 fleas were recovered from the above cage and rat (203 from the cage and 9 from the rat) at 12 noon. Of these, 162, including the 9 from the rat, were put into the freshly-prepared flea-proof cage (Cage D), together with a healthy, clean tame rat (Rat D 1), and the remaining 50 (all from the cage) were put into another cage (Cage E), with another healthy, clean tame rat (Rat E 1).

11/12/09.—Rat D 1 was removed from Cage D, was carefully freed from fleas by the aid of chloroform-vapour, and was put into a separate freshly-prepared flea-proof cage by itself. 20 fleas recovered from Rat D 1 were returned to Cage D. A clean, healthy tame rat (D 2) was put into Cage D.

Rat D 1 was examined regularly from 14/12/09 until 22/1/10. No trypanosomes were found in its blood at any time.

13 12/09.—Rat D 2 was removed from Cage D, was carefully freed from fleas, and was then put into a separate freshly-prepared flea-proof cage by itself. 54 fleas recovered from Rat D 2 were returned to Cage D.

Rat D 2 was examined regularly from 16/12/09 until 22/1/10. No trypanosomes were found at any time in its blood.

14/12/09.—A healthy, clean tame rat (Rat D 3) was put into Cage D.

15/12/09.—Rat D 3 was removed from Cage D, was carefully freed from fleas, and was then put into a freshly-prepared flea-proof cage by itself. 36 or 38 fleas recovered from Rat D 3 were returned to Cage D.

Rat D 3 was examined regularly from 17/12/09 until 21/12/09, when trypanosomes were first seen in its blood. Permanent smears were then made of its blood from 21/12/09 till 26/12/09, when it was found dead before the multiplication-period had come to an end.

16/12/09.—A healthy, clean tame rat (D 4) was put into Cage D.

17/12/09.—Rat D 4 was removed from Cage D, was carefully freed from fleas, and was then put into a freshly-prepared cage by itself. 15 fleas were recovered from Rat D 4, but were all accidentally killed.

Rat D 4 was examined regularly from 20/12/09 till 22/1/10. No trypanosomes were found at any time in its blood.

18/12/09.—A healthy, clean tame rat (Rat D 5) was put into Cage D.

20/12/09.—Rat D 5 was removed from Cage D. Though carefully searched by the aid of chloroform-vapour, no fleas were found on it. Rat D 5 was put into a freshly-prepared cage by itself. A healthy, clean tame rat (Rat D 6) was then put into Cage D.

Rat D 5 was examined regularly from 21/12/09 till 22/1/10. No trypanosomes were found at any time in its blood.

22/12/09.—Rat D 6 was removed from Cage D, was carefully freed from fleas, and was then put into a separate freshly-prepared cage by itself. 4 fleas recovered from Rat D 6 were returned to Cage D. A clean, healthy tame rat (Rat D 7) was then put into Cage D.

Rat D 6 was regularly examined from 23/12/09 till 22/1/10. No trypanosomes were found at any time in its blood.

24/12/09.—Rat D 7 was removed from Cage D. Though carefully searched by aid of chloroform-vapour, no fleas were found on it. Rat D 7 was then put into a freshly-prepared cage by itself. A healthy, clean tame rat (Rat D 8) was then put into Cage D.

Rat D 7 was examined on 25/12/09 and was found dead on 26/12/09.

28/12/09.—Rat D 8 was removed from Cage D, was carefully freed from fleas, and was put into a separate cage by itself; one flea recovered from it was returned to Cage D. A healthy, clean tame rat (Rat D 9) was then put into Cage D.

Rat D 8 was regularly examined from 29/12/09 till 3/1/10, when trypanosomes were first seen in its blood. Permanent smears were then made from 3/1/10 till 8/1/10, when the multiplication-period came to an end.

3/1/10.—Rat D 9 was removed from Cage D, was carefully searched for fleas, but no flea was found on it and it was put into a separate cage by itself. A healthy, clean tame rat (Rat D 10) was then put into Cage D.

Rat D 9 was regularly examined from 1/1/10 till 4/1/10, when trypanosomes were first seen in its blood. Permanent smears were then made from 4/1/10 till 10/1/10, when the multiplication-period came to an end.

(Experiment still proceeding—see below.)

Remarks.—It should be noted that on some occasions considerable numbers of fleas were recovered from the experimental rats in Experiment D, *e.g.*, 54 fleas on December 13, 38 fleas on December 15. As these fleas were in all cases chloroformed in order to get them off the rats, it is possible that some of them may not have recovered from the chloroform when returned to Cage D, but may have succumbed; if so, the number of fleas active in this experiment would have diminished as the experiment proceeded. This and the short time during which some of the experimental rats were exposed may account for the break in the series of infections. Allowing for differences in individual susceptibility, it may be that the chances of any given rat becoming infected increase, up to a certain limit, with each bite of an infective flea. It must be pointed out, further, that the fleas were exposed to infection for only 22 hours. As they had been kept without food for 24 hours previous to this, it is probable that at least the greater number of fleas that survived must have fed on the infected rat. Comparing the results of Experiment D with the almost unbroken series of infections in Experiment C and with the positive results of Experiment A, and allowing for all possible errors, we may conclude that the chances of the flea becoming infective increase, up to a certain limit, with the time during which it is exposed to infection. It may be that only certain individual trypanosomes in the blood are capable of producing a permanent infection in the flea.

From Experiments C and D, it would appear that bred clean fleas that have fed on a rat heavily infected with *T. lewisi*, and showing many trypanosomes in its blood, are not infective until at least six days, or more, have elapsed from the time of their having first fed on infected blood; but that from the seventh day onwards they may retain the infection, as is most clearly seen in Experiment C, so as to be capable of infecting a series of clean, healthy rats, without themselves being exposed to re-infection. It is possible, even probable, that with less heavily infected rats and with varying conditions of temperature, season, etc., the incubation-period in the flea may vary somewhat, and that six days may be about the minimum period required for the completion of the cycle in the flea. In Experiments A and B this period seems to have been exceeded, although these two experiments deal, strictly speaking, with the method of infection without special attention to the length of the incubation-period in the flea. In none of our experiments, neither in those recorded here nor in any others we have performed, has direct infection taken place. In the case of *T. lewisi*, it is very doubtful if infection by the direct method ever does take place in nature, and even when conditions are favourably arranged in experiments, infection by the direct method has not been proved. Nuttall's experiments with fleas, to which reference has been made, were concerned with the *fact* of transmission only, and were not designed in such a way as to allow of any conclusion being drawn as to the *method* of transmission. He records a positive experiment with *Ceratophyllus fasciatus*, and two positive experiments with *Ctenophthalmus agyrtes*. The fleas were in every case taken from infected wild rats, without any knowledge of when they first had an opportunity of feeding on infected blood. From such experiments it is useless to speculate on the method of infection. Nuttall, in the same paper, records a positive result with the rat-louse *Hæmatopinus spinulosus*, and concludes—"Since three distinct kinds of blood-sucking insects are capable of transmitting *T. lewisi*, it appears doubtful that this flagellate is a parasite of the invertebrate host in the sense claimed by Prowazek and other investigators." From the standpoint of transmission, however, the most important consideration in the general trypanosome problem is the distinction between "direct" and "cyclical" infection, which we laid down and defined above; and from the experiments which form the subject of the present communication, we conclude that the rat-flea *Ceratophyllus fasciatus* transmits *T. lewisi* from infected to non-infected healthy rats by the "cyclical" method, and that transmission by the "direct" method has not taken place. The importance, both from the scientific and the practical standpoint, of this "cyclical" method of infection cannot be over-estimated.

The length of the multiplication-period in the rat is probably less dependent on external conditions, and appears to be fairly constant. From these experiments, 12 days may be taken as the average length of the period from the time of inoculation by the flea until the multiplication ceases of the trypanosomes in the rat's blood. On the other hand, Experiment D defines the incubation-period in the flea within narrow limits: at the most from December 7 to 15, *i.e.* eight days, at the least from December 8 to 14, *i.e.* six days. From some of our other experiments, however, it would appear that in some cases the incubation-period is much longer, and that the cycle takes longer to complete in some fleas, or under some circumstances which are perhaps related to the conditions of the trypanosomes when taken up by the flea.

With regard to the cycle of development which the trypanosomes undergo in the flea, we hope to return to this in a future communication, but in the meantime we may point out from the observations recorded above of fleas dissected, that multiplication probably starts in the rectum of the flea, where masses of *Crithidia*-like forms are seen attached to the walls of the gut between the large rectal glands so-called. That these forms are stages in the development of *T. lewisi* in the flea is supported by the fact that in 50 fleas from the breeding cages dissected and examined, no trace of any flagellates was seen, whereas of 24 fleas from the same source, dissected after they had fed upon rats infected with *T. lewisi*, flagellates were found in 15, in 8 of which *Crithidia*-like forms were found in the rectum.

III. *Conclusions to be drawn from the Experiments.*

(1) The rat-flea *Ceratophyllus fasciatus* can transmit *Trypanosoma lewisi* from infected to non-infected rats.

(2) The transmission takes place by the cyclical method.

(3) Transmission by the direct method has not been proved to occur.

(4) The incubation-period of the flea, that is to say the period occupied by the developmental cycle of the trypanosome, has a minimum length of six or seven days, but may be longer.

(5) The multiplication-period of the trypanosome in the rat has a length of about 12 days.

(6) In the developmental cycle the establishment of the trypanosome in the flea begins with multiplication of *Crithidia*-like forms in the rectum.

Our conclusions with regard to the method of infection by the invertebrate host agree in the main with the results of Kleine's investigations upon the transmission of Sleeping Sickness of man, and Tsetse-fly Disease of animals, by *Glossina palpalis* and *G. morsitans* respectively.

Postscript.—January 24, 1910. *Tabular Summaries of Experiments C, D, and E.*

Experiment C.

Cage C colonised with 160 fleas that had been exposed to infection from November 24 till November 27.

Rat.	Put in.	Taken out.	Result.	Trypanosomes first seen.	Multiplication ended.
C 1	November 27	November 30	0	—	—
C 2	,, 30	December 3	+	December 10	December 15
C 3	December 3	,, 6	+	,, 13	,, 18
C 4	,, 6	,, 8	+	,, 13	,, 19
C 5	,, 8	,, 10	+	,, 14	,, 21
C 6	,, 10	,, 11	+	,, 15	,, 23
C 7	,, 11	,, 13	+	,, 17	,, 24
C 8	,, 13	,, 15	+	,, 20	,, 26
C 9	,, 15	,, 17	+	,, 21	Found dead on December 28
C 10.........	,, 17	,, 20	+	,, 26	December 30
C 11.........	,, 20	,, 22	+	,, 28	January 1
C 12.........	,, 22	,, 24	Found dead on December 26.		
C 13.........	,, 24	,, 28	+	January 3	,, 8
C 14.........	,, 28	,, 31	0	—	—
C 15.........	,, 31	January 3	+	January 8	January 13
C 16.........	January 3	,, 6	+	,, 10	,, 15
C 17.........	,, 6	,, 10	+	,, 12	,, 17
C 18.........	,, 10	,, 13	+	,, 19	,, 24

Experiment D.

Cage D colonised with 162 fleas that had been exposed to infection from 2 p.m. on December 7 to noon on December 8, *i.e.* for a period of 22 hours only.

Rat.	Put in.	Taken out.	Result.	Trypanosomes first seen.	Multiplication ended.
D 1	December 8	December 11	0		
D 2	" 11	" 13	0		
D 3	" 14	" 15	+	December 21	Rat died 26/12/09 before multiplication ended.
D 4	" 16	" 17	0		
D 5	" 18	" 20	0		
D 6	" 20	" 22	0		
D 7	" 22	" 24	0		
D 8	" 24	" 28	+	January 3	January 8
D 9	" 28	January 3	+	" 4	" 10
D 10.........	January 3	" 6	0	—	—
D 11.........	" 6	" 10	+	January 13	January 19
D 12.........	" 10	" 13	+	" 17	" 23

Experiment E.

Cage E colonised with 50 fleas that had been exposed to infection from 2 p.m. on December 7 to noon on December 8, a period of 22 hours. (See account of Experiment D.)

Rat.	Put in.	Taken out.	Result.	Trypanosomes first seen.	Multiplication ended.
E 1	December 8	December 11	0	—	—
E 2	" 11	left in	+	January 10	January 15

Remarks on Experiment E.—Allowing 12 days for the multiplication-period in the rat, we arrive at January 3 as being the date on which Rat E 2 became infected. The length of the incubation-period in the flea would thus appear to have been 26 or 27 days in this experiment, but this long incubation-period is, in our opinion, capable of a different explanation. We know that fleas once infective retain infection, and as Rat E 2 was left in Cage E, we may suppose that, being comparatively immune to begin with, it withstood infection for a long time, but that at last its resistance was overcome. That some tame rats are resistant in this way is shown by the breaks in Experiment D, and perhaps even more strikingly by the single break (Rat C 14) in the series in Experiment C.

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On the Relative Sizes of the Organs of Rats and Mice bearing Malignant New Growths.

By Dr. F. MEDIGRECEANU (Bucharest).

(Communicated by Dr. J. Rose Bradford, Sec. R.S. Received December 21, 1909,
—Read February 3, 1910.)

(From the Laboratory of the Imperial Cancer Research Fund.)

The object of the present investigation has been to determine the effects of the growth of tumours on the weight of the principal organs of the body. The fundamental conception on which the work is based is that the weights of the different organs of normal animals bear a relatively constant ratio to the total weight of the body.

Such investigations are capable of throwing light on many debatable points of cancer metabolism, and give important indications of promising directions for future more detailed work. The previous investigations of workers in the Imperial Cancer Research Fund (Cramer, Haaland, Murray), and recently by Moreschi, in Ehrlich's Institute, have dealt with this subject from the standpoint of the ratio of tumour-weight to total body-weight and the influence of the former on the latter and on the normal growth of the body. In the present investigation a closer analysis of the factors is attempted, in reasonable expectation that under the influence of the physical and chemical changes taking place in the bodies of animals bearing tumours, definite aberrations from the normal relations may be produced. From a consideration of these aberrations it may be possible to infer the nature of these changes.

Method.

The following precautions have been taken to avoid the sources of error which would impair the comparability of the numerical data. Normal and tumour-bearing animals (tumour-animals) were chosen which were free from obvious illness, looked healthy and strong even when bearing large tumours. They were killed by fracture-dislocation of the cervical vertebræ. Estimations were made on 300 animals. With few exceptions the mice were taken three at a time, the rats singly. In all cases the comparisons are made with total body-weight *less the contents of the alimentary canal.*

The abdominal organs were removed in the order—spleen, alimentary canal, kidneys, liver. The thorax was then opened, blood-clots removed, and lungs and heart excised. The heart was separated from the lungs and the blood-clots removed from its cavities. The unopened alimentary canal was first weighed, then split open throughout its length and washed with 0·75-per-cent. salt solution till the wash-water remained clear. It was then dried rapidly between filter-paper. All the organs were weighed in closed weighing bottles, after which the moisture was driven off in a drying oven kept at 103° to 105° C. for 40 to 50 hours, and the weight of the dry substance determined. The tumour weights include the fluid and necrotic parts as well as the growing parenchyma. Hence, in comparison with the results obtained from normal animals, especial weight must be laid on the *ratios* of the weights of organs to *body-weights minus tumour.*

Normal Ratios.

A large number of normal mice belonging to different age-periods were examined. The variations due to age are most marked when the animals pass from a milk (sucking) to a vegetarian (adult) diet. The alimentary canal is relatively twice as heavy in animals a few days after the establishment of vegetarian diet as at nine days (milk diet). During the remainder of life the ratio remains fairly constant with a slight diminution in old age. Nearly identical variations are presented by the liver. Heart and lungs are, on the contrary, relatively larger in sucking mice. The kidney shows only small variations, whether we compare animals of different ages or individuals of the same age. The spleen varies enormously, but is relatively smaller in sucking and young animals than in adults. It follows that comparisons are only allowable between animals of the same age. Equally extended observations have not been considered necessary in the case of rats, and in these the control observations have been restricted to normal animals of the same weight as the tumour-animals. Considerations which will be fully dealt with in a later paper led me to undertake experiments in which rats were fed with a uniform diet (150 c.c. milk, 350 grammes rice or oatmeal, and 60 grammes bread) for several weeks or months. The weight-ratios of some organs were found to be modified by this diet, *e.g.*, those of the alimentary canal, heart, and kidneys were relatively smaller, that of the lungs larger, and that of liver unaltered. A number of tumour-rats were fed on the same diet, and the control estimations are taken from normal rats correspondingly fed. In the same way the tumour-rats fed on the ordinary laboratory diet are compared with similarly fed normals.

Ratios in Tumour-animals.

Alimentary Canal.—The general impression of all the estimations has been that, under the experimental conditions thus far investigated, the relative weight of the alimentary canal to "body-weight less tumour" falls within or near the normal ratio. It is remarkable that in most cases (*cf.* the figures for mouse tumours, Table I, and for rat tumours, Table II), the relative weight of the alimentary canal diminishes with the size of the tumour, whether the latter is carcinoma or sarcoma, growing rapidly or slowly. Only in 8 to 10 out of 200 cases, and then especially in animals with small tumours, did the weight ratio of the alimentary canal reach the highest physiological value. A hypertrophy of this organ-system could not be demonstrated during the several stages of growth studied for the tumours examined.

Liver.—The liver, on the contrary, appears hypertrophied in all cases when the ratio to "body-weight less tumour" is taken. This holds for spontaneous as well as transplanted tumours. A direct parallel subsists, up to a certain

Table I.

No.	Age.	Ratio: tumour-weight to body-weight.	Digestive tract.	Liver.	Heart.	Kidneys.	Lungs.	Spleen.
Normal Mice.—Ratio of Organ-weight to Body-weight.								
12	51 days	—	1 : 20·8	1 : 13·8	1 : 148·4	1 : 60·9	1 : 119·3	1 : 81·4
21	3 mos.	—	1 : 20·4	1 : 12·7	1 : 157·8	1 : 57·9	1 : 120	1 : 54·4
18	5 ,,	—	1 : 23·3	1 : 14·4	1 : 168	1 : 53·9	1 : 121·5	1 : 73·3
24	Old	—	1 : 22·7	1 : 13·7	1 : 159·1	1 : 53·9	1 : 113	1 : 80·7
Pregnant Mice.—Ratio of Organ-weight to Body-weight less Fœtus.								
4	—	—	1 : 24·5	1 : 10·5	1 : 160·6	1 : 60·4	1 : 123·1	1 : 111·1
Mice with transplanted Tumours.—Ratio of Organ-weight to Body-weight less Tumour.								
A. Rapidly-growing Strains.—"63," "J," "B," "199."								
33	3–4 mos.	1 : 2·3	1 : 22·1	1 : 9·9	1 : 116·1	1 : 55·7	1 : 96·7	1 : 43·6
43	3–4 ,,	1 : 3·3	1 : 21·2	1 : 11·0	1 : 138·1	1 : 56·5	1 : 101·8	1 : 62·4
B. Intermediate Strains.—"50," "39," "100," "92."								
10	3–5 ,,	1 : 2	1 : 21·7	1 : 10·1	1 : 118·7	1 : 53·3	1 : 83·4	1 : 53·3
27	3–5 ,,	1 : 3·6	1 : 19·8	1 : 10·1	1 : 125·7	1 : 52·8	1 : 110·3	1 : 48·9
C. Slowly-growing Strains.—"62," "T," "93," "173."								
9	3½–6 ,,	1 : 1·7	1 : 20·0	1 : 9·2	1 : 128·8	1 : 54·1	1 : 119·5	1 : 50·3
27	3½–6 ,,	1 : 4·0	1 : 18·3	1 : 10·9	1 : 123·3	1 : 53·0	1 : 108·1	1 : 35·5
Mice with Spontaneous Carcinomata.								
1	Old	1 : 1·4	1 : 25·6	1 : 8·9	1 : 114·5	1 : 53·8	Metastases	1 : 56·3
1	,,	1 : 3·5	1 : 25·1	1 : 7·3	1 : 128·1	1 : 61	,,	1 : 40·2
1	,,	1 : 3·6	1 : 23·2	1 : 9·9	1 : 101·4	1 : 56	,,	1 : 54·7
1	,,	1 : 3·6	1 : 23·5	1 : 10·6	—	1 : 55	,,	1 : 44·1

Table II.

No.	Age.	Ratio : tumour-weight to body-weight.	Digestive tract.	Liver.	Heart.	Kidneys.	Lungs.	Spleen.
Normal Rats.—Ratio of Organ-weight to Body-weight less Tumour.								
A. Usual Diet.								
5	2–4 mos.	—	1 : 19·7	1 : 19·9	1 : 180	1 : 94·6	1 : 140·3	1 : 132·4
B. Special Diet.								
9	2–4 ,,	—	1 : 30·3	1 : 19·9	1 : 205·9	1 : 122·7	1 : 102	1 : 114·7
1—2 Days after Litter.								
2	4½ ,,	—	1 : 29·8	1 : 16·6	1 : 211·8	1 : 109·4	1 : 114·4	1 : 148·7
Negative Rat (20 days after the Tumour had disappeared).								
1	4 ,,	—	1 : 29·3	1 : 15·6	1 : 198·8	1 : 103·7	1 : 74·8	1 : 145
Rats with Transplanted Tumours.								
Sarcoma.								
A. Usual Diet.								
2	3½–5 ,,	1 : 1·6	1 : 24·7	1 : 11	1 : 134·8	1 : 62·4	1 : 116·3	1 : 54·4
6	3–4½ ,,	1 : 3·6	1 : 24·2	1 : 13·2	1 : 158·6	1 : 67·2	1 : 110·6	1 : 115·1
B. Special Diet.								
7	3–5 ,,	1 : 2	1 : 28·9	1 : 12·1	1 : 138	1 : 61·1	1 : 99·4	1 : 101·4
Carcinoma.								
Usual Diet.								
3	4–4½ ,,	1 : 8·2	1 : 24·8	1 : 14·0	1 : 200·2	1 : 93·0	1 : 95·8	1 : 67·6

point, between the growth of the tumour and the weight of the liver, whether comparison is made between different tumours of the same strain, tumours of different strains, or spontaneous tumours. In a single case, and under exceptional circumstances, an aberrant result was obtained. A mouse with a tumour five months old, of strain "37," weighed 19·5 grammes, while its tumour weighed 22·5 grammes. The liver was of normal weight; it is not possible to exclude the possibility that a stage of hypertrophy had been passed through. Since this case stands alone, undue weight cannot be laid upon it.

The ratio of liver to "body-weight plus tumour" corresponds in the majority of cases with the normal values. Frequently, however, one finds, on the one hand, as *e.g.* with the rat-tumours (and also Tumour "39"), that the liver is too large in proportion to "body-weight plus tumour"; on the other hand, especially when the tumours are very large, the ratio of liver to "body-weight plus tumour" is smaller than the normal value. The weight of the tumour usually includes an indeterminate quantity of dead material (necrosis and fluid), as already pointed out, so that more accurate comparisons are made with "body-weight less tumour," and on this basis the liver is always enlarged.

The constancy of this result makes a more detailed discussion desirable. It is necessary to be sure that the disturbance of the normal ratio is not only apparent and due neither to mere loss of weight in the other organs, nor to the liver reaching a weight equivalent to what it would have attained in the same animal during the natural augmentation of the body-weight. The first of these two possibilities is easily excluded. The liver is heavier than normal, even if the estimated loss in weight be added to the body-weight. One example may suffice. The quota contributed by the tumour to the total weight (body plus tumour) was calculated in a rat with transplanted sarcoma from daily weighings of the animal, and weekly estimations of the growth of the tumour. It was found that the maximum body-weight was 132 grammes 12 days before the animal was killed. The liver weighed 10·5 grammes at death. The ratio 10·5 : 132 : : 1 : 12·5 is higher than the normal 1 : 19·9. The tumour in this case weighed 55·7 grammes, and if we assume that this were merely a part of a normal animal, the total (rat plus tumour) reaches 180 grammes. The contents of the alimentary canal weighed 12·6, which, deducted from the gross weight, 180 grammes, gives 167·4 grammes, and thus the liver ratio 1 : 15·9 is higher than normal. Loss of body-weight does not introduce a disturbing factor in the majority of the cases examined, since most animals presented the same weight as at the time of inoculation. In some cases the body-weight had increased, and only in a few cases had it diminished. The differences in liver-ratio are, however, considerable, so that the second possibility can also be excluded. A rat was examined 20 days after complete disappearance of a transplanted sarcoma which had reached a size of 10 to 12 grammes. The liver was found to be enlarged still (*vide* Table II). This case should be compared with the results obtained in pregnant mice in which a hypertrophy of the liver was also noted (*vide* Table I); apparently this hypertrophy disappears in a short time after the birth of the young. There are, however, other differences between the livers of normal and tumour animals, such as the increased percentage of water in the latter (as high as 4 to 5 per cent. more than in normals), which indicate qualitative differences as well.

More detailed investigations are being made to determine when the hypertrophy of the liver commences. So far the results have been inconstant. Distinct hypertrophy was found, *e.g.* in one case with a tumour one-seventh of the body-weight, but the ratio fell within the normal in other cases for tumours of the same size.

Heart.—The heart is enlarged both in transplanted and in spontaneous tumours. In general the enlargement is proportional to the weight of the tumour (*cf.* Tumours "63," "50," rat-sarcoma, Table II). Exceptions are not infrequent. Rapidly growing tumours, *e.g.* "J," do not give so marked hypertrophy as those which grow more slowly with a richly developed blood-supply, *e.g.* "Tumours "50" and "39." The mechanical factors are in all probability mainly responsible. The pregnant normal animals examined did not present a corresponding enlargement of the heart.

Kidney.—The kidneys of tumour mice (and pregnant mice) showed only minor variations such as are met with in normal animals. The transplanted rat sarcoma, on the contrary, induced constantly an increase in the size of these organs. The enlargement could be recognised with the naked eye in animals with large tumours, and the weights were found to be double the normal expectation in many cases. This enlargement was directly proportional to the size of the tumours, as in the case of the liver and heart. The sarcomata of the Mouse "92," "37 sarcoma," "100 sarcoma," did not produce this change. Therefore it is not characteristic of the sarcomata.

Microscopical examination of the enlarged kidneys showed hyperæmia, with here and there hæmorrhages and degenerated cells. Mitoses seemed to be more frequent in the tubular epithelium than in normal kidneys.

Lung.—The results are inconstant. In one and the same strain normal and enlarged lungs are found and constant alterations are not produced.

Spleen.—As in normal animals, the spleen is subject to enormous individual variations in tumour-animals. Although exceptionally large spleens were found in many cases (*e.g.* Tumours "173," "B"), it is impossible to refer the hypertrophy to the presence of the tumours.

In conclusion, we desire to express our indebtedness to the Executive Committee and Director of the Imperial Cancer Research Fund for the facilities afforded for this investigation. It is a pleasure to acknowledge the assistance and interest of Dr. Bashford and his Assistants, Drs. Murray, Haaland, and Russell, and of Dr. Cramer.

Summary.

Weighing experiments on 200 rats and mice bearing tumours (13 transplantable and four spontaneous mouse tumours, 2 transplantable rat tumours) have shown :—

(1) No increase in weight of the alimentary canal as compared with normal ratios.

(2) Hypertrophy of the liver in all cases. Up to a certain point the hypertrophy is proportional to the weight of the tumour.

(3) Hypertrophy of the heart in most cases. This also is in general proportion to the size of the tumour.

(4) Kidneys of normal weight except in the case of a transplantable rat sarcoma in which hypertrophy is produced.

(5) Varying ratios for the lungs.

The most important result of the investigation has been the discovery of an enlargement of the liver, in animals bearing carcinomata and sarcomata, whether transplanted or naturally arising. The nature and causation of this hypertrophy is being further investigated. It would be natural to seek an explanation in an increased assimilation of food through the alimentary canal. The results show an absence of a corresponding hypertrophy of the digestive tract for tumour animals. Histological studies have not, so far, indicated any clear anatomical changes. In a future paper the hypertrophy of the liver will be considered from the standpoint of the intake and elaboration of food material from the intestinal canal, and of the excretory functions of the liver. The possibility will be considered of the circulation of abnormal products given off by the tumour, and the possibility that the liver may elaborate abnormal products to meet the special needs of the tumour.

The Contrast in the Reactions to the Implantation of Cancer after the Inoculation of Living and Mechanically Disintegrated Cells.

By M. Haaland, M.D., Imperial Cancer Research Fund.

(Communicated by Prof. J. Rose Bradford, Sec. R.S. Received January 17,—
Read February 3, 1910.)

The object of the present paper is to show the difference obtaining between the employment of the living cell, cancerous and normal, as an agent to induce active resistance to the implantation of cancer cells, and the employment for the same purpose of the protein obtained from these cells by mechanically disintegrating them. As a means of devitalising the cells with least disturbance of their chemical properties, the method adopted was that of grinding them either in the MacFadyen-Rowland apparatus at the temperature of liquid air, or in a mortar, cooled by embedding it in ice and salt. Comparison of the results obtained by both methods showed that the latter was the more efficacious. By interrupting the freezing, the material can be kept of a pasty consistence specially favourable for crushing all the cells. The completeness of the disintegration of the cells was ascertained by microscopic examination, revealing the absence of intact nuclei, and, in the case of the cancer tissue, also by inoculation of the material not yielding tumours. Every precaution has been taken to employ mice as uniform in age, size, and weight as possible, in order to make the estimations upon a soil of uniform natural resistance.

In order to analyse the results more closely in each experiment, the animals were killed after the same interval of time, when the tumours threatened to ulcerate, and all the tumours obtained were weighed. The protocol of such an experiment is given, showing all the details of the experiment, including: (1) The length of time elapsed between inoculation and the date when mice were killed; (2) number of tumour mice alive up till then; (3) total number of mice alive; (4) total weight of tumour obtained; and (5) average weight of mice. To obtain an estimate of the average growth, two calculations have been made: the one indicates the average weight of tumour obtained, calculated on all the animals of the series living up till the time when they were killed (negative included): ("average weight of tumours *pro* mouse"); the other gives the average weight of each tumour, when only the positive mice are taken into account ("average weight of tumours *pro* tumour"). It seemed inadvisable to use the figures obtained for more elaborate calculations, because of the only approximate value of all such figures obtained by biological experiment. In all transferences of cancer cells, small factors, such as health of the animals, intercurrent diseases—factors which are incapable of exact measurement—play a very important part in determining the results.

The accompanying table illustrates the nature of results obtained, after the inoculation of 0·10 c.c. of material, devitalised by grinding for 1½ hours at the temperature of liquid air, when the mice are tested 15, 17, and 20 days later, with varying doses, 0·02 to 0·05 of mammary carcinoma "63." The average weight of the tumours obtained in the treated mice when killed was 3·37, 3·2, and 4·75 grammes respectively, as against 2·03, 2·38, and 2·2 respectively in the control mice.

When the disintegration of the cells has been complete, not only does no immunity follow upon the inoculation of 0·10 to 0·50 c.c. of the disintegrated material, but in the majority of cases a distinct hypersensitiveness is induced in the animals so treated.

Varying the interval of time between the preliminary inoculation and the testing inoculation shows that this hypersensitiveness is not a phase antecedent to the establishment of immunity. There is as little evidence of acquired resistance when the animals are tested at 30 days after the preliminary treatment as there is at 10 and 20 days.

The effect of inoculating varying doses of devitalised material has been studied. When a scale of different doses ranging from 0·50 to 0·025 c.c. is inoculated, the weight of the tumours obtained from the testing inoculation shows that 0·10 c.c. gave a greater average weight of tumour than larger or smaller doses. Although it would appear that the intermediate dose of 0·10 c.c. offers optimum conditions for hypersensitiveness, even so large a dose as 0·50 c.c. does not induce any increased resistance as compared with the normal animals of the same experiment. This is a relatively enormous dose, and its inefficacy as an immunising agent shows that the absence of immunity after the absorption of dead material is not merely a question of too small a dose.

Having established that the immunising power of tumours is abolished by mechanically injuring the cells (*i.e.* with the minimal chemical alteration in them), so that no growth ensues, the question next arises if the immunity obtained by normal tissues runs the same course. Fig. 1 shows that this is the case. In this experiment different batches of mice of about the same age were treated, one set with an emulsion of fresh total mouse-embryo, another with the same total mouse-embryo emulsion, ground in a mortar at a temperature of 0° C. for 1½ hours; a third batch was treated with fresh

	Preliminary inoculation.					Interval between treatment and testing inoculation.	Testing inoculation.			Result.			Analysis of result when mice killed.						
Experiment.	Material.	How treated.	Dose.	Date.	Remarks.		Tumour.	Dose.	Date.	No. of tumours.	No. of mice.	Percentage.	Days after inoculation when killed.	No. of tumours.	No. of mice.	Total weight of tumours.	Average weight of tumours.		Average weight of mice.
																	Pro mouse.	*Pro* tumour.	
1	63 carc.	Ground at the temp. of liquid air	0·10	6.1.09	No growth	Days. 15	63 carc. (Exp. 23B)	gr. 0·02	21.1.09	11	11	Per cent. 100	Days. 32	8	9	30·30	3·37	3·8	15·99
					Controls...	...	—	—	—	8	10	80	—	8	10	20·30	2·03	2·54	18·24
2	As above	—	—	—	No growth	17	63 carc. (Exp. 24A)	c.c. 0·025	23.1.09	10	10	100	34	9	9	28·85	3·21	3·21	16·4
					Controls...	...	—	—	—	9	10	90	—	8	9	21·40	2·38	2·68	17·43
3	As above	—	—	—	No growth	20	63 carc. (Exp. 23D)	c.c. 0·05	26.1.09	8	8	100	35	8	8	38·0	4·75	4·75	17·97
					Controls...	...	—	—	—	8	9	89	—	7	8	17·30	2·2	2·47	18·5

mouse-embryo skin; a fourth with mouse-embryo skin ground at a temperature of about 0° as above. Fourteen days later all these mice, with controls corresponding, were inoculated with 0·01 c.c. of carcinoma "63." Fig. 1 shows the result of this experiment as observed on the 10th, 17th, 24th, and 31st day after the inoculation. In the control mice there are 17 tumours in 18 mice, *i.e.* 94 per cent. The mice treated with fresh embryo-emulsion

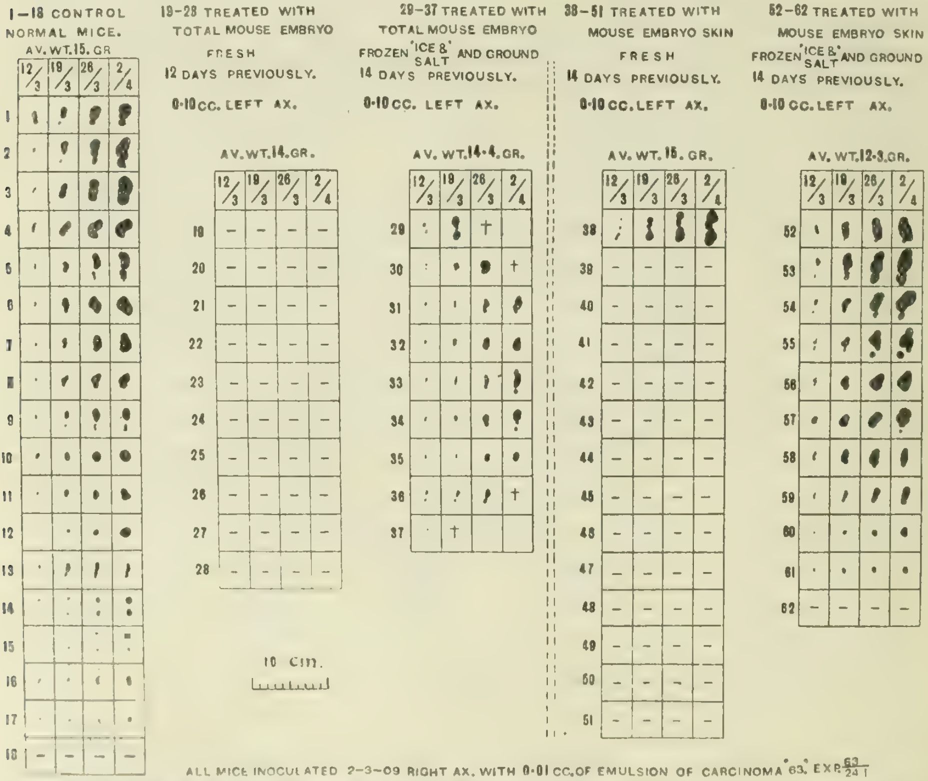

Fig. 1.—Experiment 63/24 I, showing the immunising effect of preliminary treatment of mice with normal tissues and the abolition of this effect after disintegration of the tissues by freezing and grinding. The numbers represent individual mice treated as stated at the top of each column. The silhouettes represent to scale the sizes of the tumours obtained in each animal at the dates stated, 10, 17, 24, and 31 days after inoculation. Negative results are represented by –.

are completely protected, whereas the mice of the third column, treated with ground embryo, developed tumours in each case, *i.e.* 100 per cent. The somewhat slower growth of the tumours in the third column, as compared with the controls, may be attributed to an infection, which killed several mice in the same cage, as shown on the chart. The mice treated with fresh skin gave one tumour in 14 mice (*i.e.* 7 per cent.), whereas the corresponding batch treated with skin ground at a temperature of about 0° C. gave 10 tumours in 11 mice, *i.e.* 91 per cent., and these tumours grew more rapidly than the control tumours. This experiment demonstrates that an efficient disintegration of the cells of normal tissues robs them of all immunising properties. We have later repeated this experiment with other normal tissues, spleen, liver, and blood, with the same result.

Another method for obtaining the proteids of the cells has been tried: that of obtaining the press-fluid from tumours and normal tissues by Buchner's press after disintegration of the cells by grinding in a mortar with sand. The effect of a preliminary inoculation of such press-fluid on a subsequent inoculation of cancer was studied. Injection of 0·50 c.c. of tumour press-fluid, 11 days previous to testing inoculation, far from having any immunising effect, on the contrary hypersensitises the animals.

These experiments with ground material and press-fluid, both of cancerous and normal tissues, show that the power of inducing resistance is not bound up with the proteid of the cell as a chemical entity, but in some way or other depends upon properties of the living cells.

The investigations described above refer especially to mechanically disintegrated cells, but the results also apply to disintegration by autolysis, by heat, and to the influence of radium. In this latter case the microscopical appearance of the cells and their anatomical structure remains apparently unaltered. This shows that the loss of immunising power is not bound to any special form of disintegration, but is common to all means by which the life of the cell is destroyed.

The conclusions are :—

Complete disintegration of the tumour cells robs them entirely of their immunising properties.

There is no difference between tumour cells and normal cells in this respect.

The absence of immunising power does not seem to be a question of dose of introduced material, because relatively enormous doses of dead material (*e.g.* 1/26 of the weight of the animal) do not induce any resistance, whereas minimal doses of living cells (*e.g.* 1/1300 of the weight of the animal) have this effect.

The immunising property is not bound up with the protein of the cell, but depends on a different principle. Living cells are necessary to induce resistance to transplantation of cancer. It seems necessary that these cells must not only remain alive, but also even grow for a certain time; without the fulfilment of these conditions the reaction inducing active resistance is not set up.

The reaction which introduction of disintegrated cells calls forth is not only quantitatively different from that induced by living tissues, but also qualitatively different. Far from inducing any increased resistance, inoculation of disintegrated cells only seems to manure the soil for a subsequent growth of tumours.

The failure to elicit the reactions of immunity to the transplantation of cancer by devitalised tissues reveals an important difference from the immunity reactions obtained against bacteria and their products and foreign proteids in general, in which cases the immunising properties are independent of the vitality of the organisms or cells.

Further Evidence on the Homogeneity of the Resistance to the Implantation of Malignant New Growths.

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(Communicated by Prof. J. Rose Bradford, Sec. R.S. Received January 17,—Read February 3, 1910.)

This paper is based mainly on a study of the processes at the site of implantation of a malignant new growth when a secondary implantation is practised on mice already bearing transplanted tumours. New evidence will be adduced supporting the view that concomitantly with the establishment of such tumours, an active resistance may be induced by the absorption of tumour tissue. When a secondary inoculation fails, this failure is due to an active resistance to the cancer cells introduced, similar to that induced in normal animals by the absorption of tumour tissue or normal tissue of the same species. The process consists essentially in the cancer cells failing to elicit the specific connective tissue and vascular scaffolding necessary to their establishing themselves and growing into a tumour. In order to simplify still further prevailing conceptions of the process of immunity to cancer, we shall record corresponding observations on rats where the primary inoculation of a mouse carcinoma has been followed by a secondary inoculation of mouse tumour.

In the First Scientific Report of the Imperial Cancer Research Fund (March, 1904) it was stated "in the light of the phenomena of immunity, it is interesting to note that it is possible to obtain multiple tumours from transplantations performed on the same date, and that transplantation can be successfully performed in animals in which tumours have already developed 14 days to 10 weeks after the first effective transplantation, *i.e.*, both when the primary tumour is small and when it has attained a large size." These positive results appeared to be important, first, because of their correspondence with the dissemination and formation of metastases in the normal course of the progress of cancer in man, and, secondly, because they afforded a basis for studying the conditions, favourable and unfavourable, to the establishment of secondary implantations in animals already bearing tumours, and hence had also an indirect bearing on the control of natural metastases. Therefore, these observations formed one of the starting points of our studies on the induction of resistance to the inoculation and growth of cancer. Their importance was enhanced later by the success attending efforts to reproduce experimentally both the local infiltrative and the disseminated lesions of the disease.

Other investigators, who, at a later date, were not so successful, either in re-inoculating animals already bearing tumours, or in reproducing the lesions of dissemination, have quite naturally drawn conclusions opposed to those drawn by us; for example, in April, 1906, Ehrlich reported "If metastasis formation be imitated experimentally by re-inoculating animals 8 to 10 days after they had been successfully inoculated with a rapidly growing tumour, then the second inoculation, whether it be made with the same or with a different tumour, does not take with few exceptions" (1). Ehrlich was led to attach enhanced importance to his observations by the fact that metastases were rare, or, if present, only of microscopical size, in his inoculated animals. He sought an explanation common to the two groups of observations in the assumption of "atreptic" immunity, meaning thereby that the rapidly growing tumour prevented successful re-inoculation and the establishment of metastases by withdrawing special nutritive substances (Substance X) from the circulation. The idea of "atreptic" immunity has been extended to explain also the transitory growth of mouse tumours in rats by assuming that growth ceased when the hypothetical Substance X, introduced with the graft, was exhausted. Ehrlich (2) modified his standpoint in 1908 in so far as to admit that re-inoculation may be successful although he maintains that the secondary tumours remain smaller than the corresponding tumours in control animals, and therefore he adheres to his assumption of the existence of an "atreptic" immunity.

The foregoing summary shows that the elucidation of the nature of active resistance to cancer has been complicated both by contradictory observations and by conflicting explanations of facts, regarding which there is complete agreement. These difficulties are associated more especially with the results following upon the secondary inoculation of animals already bearing tumours as the result of primary inoculation. Some authors have succeeded where others have failed to obtain secondary tumours in this way. The methods by which secondary inoculations can be successfully carried out, the importance of dosage,* and other technical details, have been fully explained in a series of papers, and the contradictory results harmonised (3, 4, 5). It is, however, necessary to meet hypothetical explanations of the reasons why a secondary inoculation may fail, by recording the results of actual observation of the processes responsible and comparing them with those following the induction of active resistance in normal animals. Therefore, the histological details of the process have been ascertained by examining the site of re-inoculation at definite intervals. This method is conveniently called the examination of "early stages."† By its means the true nature of the transplantation of cancer was demonstrated (6, 7), and later it revealed how important for the implanted cancer-cells was the provision of a specific supporting and nutritive scaffolding by the tissues of the new host (8). It also demonstrated, that in mice rendered resistant to carcinomata, there was a failure to supply this specific scaffolding, and hence the conclusion was arrived at that actively resistant animals robbed the cancer-cells of the chemiotactic powers they exercise on the connective tissues of the host (4). This failure of the specific stroma reaction was seen both when resistance was induced by the previous absorption of tumour tissue, and by the previous absorption of normal tissue—whether adult or embryonic—of the same species. For the elucidation of the mechanism of resistance there is, at present, no alternative to actual observation of the site of the inoculation of cancer-cells in living animals. Without resort to this laborious method the hypothetical assumption of an atreptic immunity was advanced to explain why a secondary inoculation may fail in an animal already bearing a tumour. By employing it the facts recorded in this paper have been elicited; they harmonise with what has been stated elsewhere on the successful re-inoculation of animals already bearing tumours, and show that the resistance which may exist to re-inoculation under these circumstances is identical in its nature and mechanism with that which can be induced in normal animals, *i.e.*, in the absence of a tumour.

It is immaterial which of the 65 tumour-strains growing in the laboratory is used to illustrate the facts, since they agree for all the strains that have been tested, whether slowly or rapidly growing. Some tumour-strains illustrate a larger number of the sum total phenomena than others. Thus, strain "199" is very suitable for studying the relations existing between a growing tumour and the host bearing it. This tumour, an adeno-carcinoma of the mamma, has now been propagated for 15 months, and gives from 70 to 100 per cent. of tumours when inoculated into a fresh batch of mice. The inoculated tumour-cells proliferate very rapidly, so that, when the strain is in a positive phase of growth, from a dose of 0·05 gramme, tumours weighing about 1·5 grammes may be obtained after 10 days, in all the mice inoculated. The subsequent behaviour of the individual tumours of the same series is very variable; about a third of them will continue to grow rapidly during the next two to three weeks; another third will show a very much slower speed of growth for two to four weeks, and then resume a speed of growth somewhat slower than the original speed of proliferation; the remaining tumours will be gradually absorbed and disappear after 3 to 5 weeks. A single series of daughter tumours of this strain "199" illustrates the extremes of behaviour presented by other tumours, viz., those growing progressively and rapidly in all animals inoculated, and those exhibiting transitory proliferation only, even when implantation is successful in 100 per cent. of the animals inoculated. The parts played respectively by active resistance induced concomitantly with the development of the tumours, and by the qualities of the tumour-cells themselves in determining the different behaviour of different tumours have already been fully discussed (4, 5).

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* It is impossible to compare the rate of growth of the tumours following inoculation if the amount of material inoculated as the starting point of growth is not stated. This factor still continues to be neglected, even in the most recent investigations of tumour growth. *Cf.* Moreschi, 'Zeitschrift f. Immunitätsforschung,' 1909.

† Fully described in the Scientific Reports of the Imperial Cancer Research Fund.

Advantage may be taken of the behaviour of tumour "199" to obtain a concise survey of the nature of the resistance or immunity against the re-inoculation of cancer in animals already bearing tumours. Series of mice, usually 20 in number, bearing growths 10 days old of tumour "199" in the right axilla, have been re-inoculated in the left axilla with another tumour either of the same strain or of another strain. Briefly, the result has been that the re-inoculation has failed to give rise to a tumour except in the cases where the tumour primarily inoculated has continued to grow rapidly. Where the primarily inoculated tumours have slowed up in their speed of proliferation, or where they have begun to diminish in size, the result of the re-inoculation has been that no tumours developed. In other words, the better the growth of the primary inoculation, the more favourable are the chances for a tumour developing from the second inoculation. This result confirms those previously recorded from this laboratory (5). The possibility can be excluded of the degree of hindrance to secondary inoculation being in direct proportion to the rate of growth of the primary tumour.

Investigation has also been made of the details of the processes at the site of the re-inoculation in mice 10 to 12 days after the primary inoculation of tumour "199." It has been found that there is an inhibition of the stroma reaction in those mice whose primary tumours had showed a slowing up in their speed of growth, or had begun to diminish in size. A study of the actual mechanism of resistance to re-inoculation points unambiguously to its being due to the induction of an active resistance to the cancer cells identical with that described for normal animals (4). It is possible for a mouse bearing a growing tumour to be actively resistant to re-inoculation, a fact which has already for other reasons been attributed to concomitant immunisation arising from absorption of a portion of the already established tumour (9). The successful re-inoculation of mice bearing the most rapidly growing "199" tumours is easily understandable on this basis, as in this case the amount of absorption of tumour tissue is practically *nil*, and resistance has not been established. The assumption of a special "atreptic" immunity in the case of animals already bearing tumours, where the host is passive and the tumour active, by withdrawing food, to explain the failure in certain cases to re-inoculate an animal the bearer of a growing tumour, is superfluous.

An immunity developing when cancer of one species is inoculated into another species of animal has been assumed by some workers to be an immunity against cancer. Only the most rapidly growing of mouse tumours when inoculated into rats grow for six to eight days according to Ehrlich (10), quite as well as in mice, but after this date they become absorbed, and the animals are then actively immune. If removed at the height of their development from the rats and inoculated into mice or rats, Ehrlich states they grow in the former but do not grow in the latter. The temporary growth in rats is explained by Ehrlich as another instance of atreptic immunity due to the absence in the rat of an unknown specific substance necessary for the growth of mouse tumours. The transitory growth of mouse tumours in rats has been shown to be common to those of slow growth (4) as well as of rapid growth, including Jensen's carcinoma, for which the possibility was denied. In the third scientific report of the Imperial Cancer Research Fund, the nature of the tissue reaction was demonstrated when rats, inoculated 14 days previously with mouse tumour, were re-inoculated, *i.e.*, while they were actively immune against mouse tumours. Further, this immunity was shown to be of a different character from the immunity of mice against mouse tumours. When rats immunised with mouse tumours were inoculated with "early stages" of a mouse tumour, there took place an active destruction of the introduced tumour graft. After two to three days there was scarcely a single cancer cell left, whilst the surrounding rat tissues were proliferating actively and invading the dying graft. Here one had to deal with an active immunity against a foreign protein, and not with an immunity against a cancer cell as such. In spite of the observations made and recorded at that time, von Dungern and Coca (11) have since compared a similar phenomenon with the immunity against cancer from which it is so totally different, and have built up an hypothesis of "allergetic" immunity against cancer. They performed their immunity experiments with an epidemically occurring fibrocellular growth of the hare, which they have succeeded in transferring through eight generations in a strange species, viz., in rabbits. They find that when a rabbit has been successfully inoculated with this growth, it is impossible to obtain success with a re-inoculation. The site of re-inoculation is frequently the seat of a marked œdema, and they have found by histological examination of the site two or more days later, that there is extensive reaction with production of large mononuclear cells which are specially abundant in the veins and capillaries. They regard this reaction as being evidence of a tissue immunity, and especially of the nature of a local hypersensibility, in that the tissues having been once exposed to the action of these foreign cells, they react more energetically to second inoculation of them, apparently as the rat tissues have been described to do to re-inoculation of mouse tumour. Von Dungern and Coca have not made the direct observation that, in transferring this hare tumour to rabbits, they have been effecting a transplantation. If we accept their view, based upon indirect observations, that the transference of this tumour is really a transplantation, then their immunity results are comparable to those obtained in rats when inoculated with mouse tumour, with this difference, that it has been possible to propagate the hare tumour through several generations of rabbits. In a recent publication, von Dungern and Hirschfeld (12) claim that this local allergetic reaction is of great importance in its bearings on immunity to cancer.

The following experiments show how very different is the nature of the immunity produced by inoculating cancer of a strange species from the resistance to cancer produced by the absorption of tumour or normal tissues of the same species. In these experiments the behaviour of tumour-strain "199" has been studied in the rat. When 0·2 c.c. of this tumour is inoculated into rats, tumours 2 to 3 cm. long by 0·5 to 0·75 cm. broad are obtained in from eight to ten days. Histological examination of these tumours at about the eighth or ninth day has shown them to be composed almost entirely of granulation tissue, which in the rat is mainly fibroblastic with a small central canal containing the inoculated tumour tissue, for the most part as necrotic *débris.* If examined at an earlier date, fifth or sixth day, small islets of carcinomatous tissue are seen lying in a very cellular tissue, the whole having the appearance almost of a carcinoma-sarcomatodes. If the tumour be removed from the rat at this earlier stage, and inoculated back into mice, it gives rise to tumours, but these do not grow so well as before *passage* through the rat, and the percentage of tumours obtained is only about a third of the previous percentage.

Nine rats, weighing 490 grammes, were inoculated on the dorsum with 0·2 c.c. of an emulsion of mouse tumour "199." Three days later, other nine rats, weighing 510 grammes, were inoculated in exactly the same way with the same tumour; and again, three days later, a third batch of nine rats, weighing 540 grammes, were treated with the same tumour in the same manner. Three days after inoculation of the last batch, that is nine, six, and three days respectively, after the primary inoculation, these three batches of rats were tested simultaneously by inoculating "early stages" of another mouse tumour in the right axilla. Twelve normal rats, weighing 455 grammes, were also inoculated with early stages to serve as control. These early stages were removed at 24, 48, 72 hours after inoculation, and examined in serial sections. In the normal control rats the "early stages" continued to grow for the first three days. The tumour cells continued to divide, whilst the introduced stroma degenerated, and was beginning to be replaced by a fresh stroma from the rat tissues on the third day. In the batch of rats treated three days previously with 0·2 c.c. of mouse tumour, no difference could be seen in the early stages as compared with the control early stages in normal rats. There was no marked difference, also, in the case of the batch treated six days previous to inoculation of the early stages. In the batch of rats which had been treated nine days previous to testing with "early stages," quite a different state of affairs obtained. In the grafts examined after 24 hours' residence, a large number of the introduced tumour cells, both in the peripheral and central parts of the graft, had been killed. Further, there was a complete absence of mitoses of the tumour cells. Mitotic division of the adjacent connective tissue cells of the rat was already present, and there was a large exudate of polymorphonuclear leucocytes. At 48 hours only a few tumour cells, mostly in the central parts of the graft, had retained their morphological characters. By 72 hours, all the tumour cells had been killed off, and an abundant reaction tissue was being formed by the rat tissues.

Such experiments demonstrate in the clearest manner that after inoculation of a given dose of mouse tumour into a rat, there is produced between the sixth and ninth day an active immunity which leads to the rapid destruction of any mouse tumour cells subsequently introduced. Further, it can be inferred that the production of this active immunity contributes to terminating the transitory growth of mouse cancer-cells in rats. The cessation of their growth is due largely to active immunity concomitantly induced, and to other influences directly injurious to them. The immunity induced in the rat against mouse cancer is very different from the active resistance of a mouse to inoculation of tumour of its own species, for, as demonstrated in 1908, mouse tumour cells can live and divide for eight to ten days in a resistant mouse, but they are incapable of eliciting the stroma reaction necessary for their establishment as a fresh tumour (4).

It has been possible to demonstrate the essential difference between these two types of immunity in another manner. Up to the present it has been found possible to render an animal resistant to tumours of its own species, only by previous inoculation of *living* tissues also of its own species. It is shown in another communication that if mouse tumour be disintegrated by pounding in a mortar cooled in salt and ice, and then be inoculated into mice, it does not give rise to the development of tumours, neither does it produce any immunity against mouse tumours; on the contrary, hypersensitiveness may result (13). Rats inoculated with a dose of 0·25 c.c. of mouse tumour ground up in this way have then been tested by introducing "early stages" of a mouse tumour. It has been found that disintegrated mouse tumour does render rats actively immune against mouse tumours. The fundamental distinction between the behaviour of disintegrated mouse tumour in mice and rats respectively, demonstrates that the immunity induced in the rats is directed not against mouse cancer *quâ* cancer, but against mouse cancer *quâ* mouse tissue, and it is exactly analogous to the anti-bodies obtained by inoculation of heterologous tissues, in the production of precipitins, hæmolysins, and cytotoxins. Indeed, corresponding reactions have been obtained *in vitro* by means of disintegrated tumour tissue inoculated into strange species (9); whereas the true reactions of induced resistance to cancer are only obtained *in vivo* by inoculation of living tumour, or tissue of the same species.

In confirmation of previous results, animals already bearing rapidly growing tumours can be re-inoculated successfully, and conclusions based upon experimental conditions permitting of the reproduction of the features of dissemination, are more worthy of consideration than conclusions based upon experimental conditions which exclude such reproduction.

The presence of an experimental tumour is compatible with the existence of active resistance to re-inoculation.

The assumption of an "atreptic" immunity to cancer has been applied to phenomena which are more naturally explained as due to an active resistance induced against cancer-cells. The assumption of a special form of immunity, "atreptic" in nature, is also incompatible with the fact that re-inoculation is successful in direct relation with the rapidity of the growth of the successful primary inoculations, and with the fact that when resistance to re-inoculation is present, it is identical with that active form of resistance induced in normal animals by the absorption of living tissue, either tumour or normal.

The assumption of an "allergetic" immunity to cancer is applied to what is really an active immunisation against the tissues of a strange species of animal.

The immunity induced in rats by the inoculation of mouse cancer, or by similar heterologous inoculations in the case of other animals, is absolutely distinct from the resistance induced against cancer of the same species to which it has no direct relation.

Only one form of active resistance to the implantation of cancer from one animal to another has as yet been demonstrated to exist. This resistance follows only upon the absorption of living tumour tissue or living normal tissue of one animal, when introduced into another animal of the same species. So far as yet elucidated, it consists primarily in an inhibition of the specific chemiotactic powers which cancer-cells exercise upon the connective and vascular tissues of the host. This single explanation harmonises all the observed facts, and should rid the experimental study of cancer both of confusion and error. It may be pointed out that investigations, such as those described in this paper, bear upon the nature and biological behaviour of cancer-cells; but that they do not admit of any inferences as to possible methods of treating the disease.

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Page

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ON THE NATURE OF MAGNETO-CATHODIC RAYS. By H. Thirkill, B.A. (Cantab.), B.Sc. (Lond.) 324

ON THE APPROXIMATE ARITHMETICAL SOLUTION BY FINITE DIFFERENCES OF PHYSICAL PROBLEMS INVOLVING DIFFERENTIAL EQUATIONS, WITH AN APPLICATION TO THE STRESSES IN A MASONRY DAM. By L. F. Richardson. (Abstract) 335

THE INITIAL ACCELERATED MOTION OF ELECTRIFIED SYSTEMS OF FINITE EXTENT, AND THE REACTION PRODUCED BY THE RESULTING RADIATION. By George W. Walker, A.R.C.Sc., M.A. (Abstract) 336

ON THE SHAPES OF THE ISOGEOTHERMS UNDER MOUNTAIN RANGES IN RADIO-ACTIVE DISTRICTS. By Charles H. Lees, D.Sc., F.R.S. 339

ON THE PROPAGATION OF A DISTURBANCE IN A FLUID UNDER GRAVITY. By F. B. Pidduck, B.A. 347

ON THE VELOCITY OF STEADY FALL OF SPHERICAL PARTICLES THROUGH FLUID MEDIUM. By E. Cunningham, M.A. 357

ON THE FLOW OF WATER THROUGH PIPES AND PASSAGES HAVING CONVERGING OR DIVERGING BOUNDARIES. By Prof. A. H. Gibson, D.Sc. 366

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PROCEEDINGS
OF
THE ROYAL SOCIETY.

Series B. Vol. 82. **No. B 556.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page

Contributions to the Biochemistry of Growth.—The Total Nitrogen Metabolism of Rats bearing Malignant New Growths. By W. CRAMER and HAROLD PRINGLE 307

Contributions to the Biochemistry of Growth.—Distribution of Nitrogenous Substances in Tumour and Somatic Tissues. By W. CRAMER and HAROLD PRINGLE 315

The Alcoholic Ferment of Yeast-juice. Part V.—The Function of Phosphates in Alcoholic Fermentation. By ARTHUR HARDEN, F.R.S., and WILLIAM JOHN YOUNG (Biochemical Laboratory of the Lister Institute of Preventive Medicine) 321

The Causes of Absorption of Oxygen by the Lungs. (Preliminary Communication.) By C. GORDON DOUGLAS, B.M., Fellow of St. John's College, and J. S. HALDANE, M.D., F.R.S., Fellow of New College, Oxford 331

The Action of Nicotine and other Pyridine Bases upon Muscle. By V. H. VELEY, F.R.S., and A. D. WALLER, F.R.S. 333

Preliminary Note on the Origin of the Hydrochloric Acid in the Gastric Tubules. By MABEL PUREFOY FITZGERALD (Oxford) . . . 346

Studies on Enzyme Action. XIII.—Enzymes of the Emulsin Type. By HENRY E. ARMSTRONG, F.R.S., and EDWARD HORTON, B.Sc. . 349

The Development of Trypanosomes in Tsetse Flies. By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908–10) . , . . 368

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Two Shillings and Sixpence.

No. B 556.

May 10, 1910.

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*Contributions to the Biochemistry of Growth.**—*The Total Nitrogen Metabolism of Rats bearing Malignant New Growths.*

By W. Cramer and Harold Pringle.

(Communicated by Prof. E. A. Schäfer, F.R.S. Received January 25,—
Read February 24, 1910.)

(From the Physiology Department, University of Edinburgh, and the Imperial Cancer Research, London.)

The following experiments were carried out with the object (1) of studying the effect of a rapidly growing neoplasm on the metabolism of the normal animal which bears the tumour, with consideration also of the view that the new growth secretes, as has often been asserted, substances having a deleterious action on the tissues of the animal bearing the tumour; (2) of elucidating the processes determining the rapid proliferation of the cells of a malignant new growth, and the source of the nitrogenous material used by the tumour.

In order to obtain facts throwing light on these questions, we have determined the nitrogenous metabolism in three rats before and after implantation of a rapidly proliferating malignant new growth. So far as we are aware, no such experiments have been made before.

The tumour used for these experiments was a spindle-celled sarcoma of very rapid growth, obtained from the Imperial Cancer Research Fund (tumour J. R. S.). This tumour can be transplanted with a high percentage of success in young animals of 40 to 60 grammes weight; with older rats of over 100 grammes weight the percentage of progressively growing tumours is not so high, and preliminary transient growth occurs more frequently. Since young growing rats did not appear to be suitable for a metabolism experiment in which it was desirable to obtain a constant nitrogen output, we found it necessary to use older animals.

Three rats of about the same age and weight were kept on a constant diet of a uniform composition. The diet used consisted of 56 grammes of stale bread made into a pulp with 50 c.c. of milk. Of this pulp 40 grammes were given to each animal in two daily rations. When a constant nitrogen output had been obtained, the rats were inoculated with a measured dose (0·1 c.c.) of the tumour, and the observations continued for the succeeding 15 days. At the end of the first week tumours could be distinctly felt. On the 16th day after transplantation the rats were killed. One animal (Rat I) had developed a large, progressively growing tumour (5·1 grammes). The second rat (Rat II) had a smaller tumour (weighing 1·55 grammes) which was not increasing in size, and which possibly would have undergone spontaneous absorption if the experiment had been continued. In the third animal (Rat III) only transient proliferation had taken place, and no tumour was found when the animal was killed. Rat III may therefore serve as a useful comparison while Rat I represents the conditions in an animal with a tumour following its normal course of progressive proliferation. The cages in which the animals were kept were similar to those used by Prof. Schäfer* in his experiments on feeding with pituitary substance. In these cages the urine and the fæces can be collected separately. The observations were made in periods of three days. The food was given in high, narrow beakers, fixed in the cage, a device which prevented the animals from spilling the food. The beakers were removed empty after one hour. The animals rapidly ate all the food given to them, and were in good condition throughout the experiment; but Rat III did not appear to be so lively during the last week of the experiment, and was not so hungry at the time of feeding as the others, so that in the last week the food had to be left in the cage during the whole day. We have repeatedly noticed that where this tumour is being absorbed after a rapid initial proliferation, the health of the animals appears to be affected.

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* This research is in continuation of paper in 'Roy. Soc. Proc.,' B, vol. 80, 1908, p. 263.

The food was analysed at different times during the experiment. It was found to have a constant N percentage. Six duplicate analyses gave 1·08 grammes N as the amount of nitrogen given in the food (120 grammes) during each three-daily period.

The results obtained are given in Table I.

It will be seen that the three animals continued to retain nitrogen and to increase in weight after transplantation. During the first two or three periods after the transplantation, when the absolute amount of tumour growth is slight, and when the introduced cells are being provided with a new stroma, the nitrogen retention remained on a low level and the animals did not gain in weight. But when the tumour had once established itself and was growing rapidly (Rat I, periods from 8, VII to 13, VII), the nitrogen retention rose rapidly. It will be shown that this is due not only to the formation of new tumour tissue, but to the organism of the host itself retaining nitrogen and increasing in weight correspondingly. The absorption of food from the intestine, as indicated by the nitrogen of the fæces, remained about the same. The urine was examined for creatine during the first nine days after transplantation. No creatine was present.

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* E. A. Schäfer, Croonian Lecture, "The Functions of the Pituitary Body," 'Roy. Soc. Proc.,' B, vol. 81, 1909, p. 442.

Table I.

	Rat I.				Rat II.				Rat III.				
Period.	Urine. N in grms.	Fæces. N in grms.	N-retention in grms.	Weight in grms.	Urine. N in grms.	Fæces. N in grms.	N-retention in grms.	Weight in grms.	Urine. N in grms.	Fæces. N in grms.	N-retention in grms.	Weight in grms.	N in food in grms.
19—21 VI	0·840	0·090	0·150	210	0·721	0·112	0·247	175	0·771	0·106	0·203	180	1·08
22—24 VI	0·847	0·081	0·152	220	0·706	0·125	0·249	190	0·741	0·096	0·243	195	1·08
25—27 VI	0·955	0·061	0·064	220	0·756	0·102	0·222	195	0·785	0·105	0·190	200	1·08
28 VI	TRANSPLANTATION.												
29 VI—1 VII	0·956	0·085	0·039	220	0·767	0·105	0·208	200	0·785	0·118	0·177	200	1·08
2—4 VII	0·890	0·102	0·088	220	0·805	0·066	0·209	200	0·801	0·096	0·183	200	1·08
5—7 VII	0·893	0·095	0·092	225	0·767	0·118	0·195	210	0·822	0·111	0·147	210	1·08
8—10 VII	0·813	0·071	0·196	235	0·759	0·120	0·201	210	0·808	0·118	0·154	210	1·08
11—13 VII	0·819	0·089	0·172	240	0·683	0·124	0·273	220	0·780	0·106	0·194	210	1·08
14 VII	....	....	Host Tumour	235 5	....	....	Host Tumour	218·5 1·5	....	....	....	....	No tumour.

If the malignant new growth secreted a substance of the nature of a toxin, one would expect to find at least a diminished nitrogen retention, if not an actual loss of nitrogen as the tumour increases in size. Our experiments give no evidence of the presence of a substance having a disturbing action on the nitrogenous metabolism.

The nitrogen retained during the experiment must have been allotted partly to the somatic tissue of the host on the one hand and partly to the tumour on the other. The proportion of nitrogen which goes to build up the new growth was found by determining the nitrogen contained in the whole tumour. The results of the analyses of the tumours are given in Table II.

Table II.

	Amount of tumour used for analysis in grammes.	Nitrogen found in grammes.	Total weight of tumour in grammes.	Nitrogen in total tumour in grammes.	Average nitrogen-content of total tumour in grammes.
Rat I...	1·095	0·02534	5·10	0·1180	0·1220
	1·025	0·02534		0·1260	
Rat II...	0·297	0·0084	1·55	0·044	0·0435
	0·323	0·00896		0·043	

The proportion of N used to build up new somatic tissue can be found by subtracting the N-content of the tumour from the nitrogen retention of the host *plus* tumour.

Table III gives the proportions of nitrogen which have gone to the building up of the somatic tissue of the host and to the formation of tumour tissue respectively. The results can be stated best in the form of the quotient, N-retention : Increase in weight. This quotient indicates the nitrogen value of the unit of tumour tissue, and unit of somatic tissue of the host respectively.

It is obvious that, before transplantation, the relation N-retention to increase in weight should be the same in the three rats, and as a matter of fact this was found to be the case. Since metabolism experiments on rats have not often been made, we may point out here that the constancy of the figures obtained is evidence of the reliability of our observations.

The most striking result obtained after transplantation is the fact that the quotient is smaller in the case of the new growth than in the case of the somatic tissue of the host. In other words, less nitrogen is needed to build

Table III.

	Before transplantation (9 days).			After transplantation (15 days).		
	Increase in weight in grammes.	N-retention in grammes.	N-retention. Weight increase.	Increase in weight in grammes.	N-retention in grammes.	N-retention. Weight increase.
				20·1 (total) − 5·1 (tumour)	0·59 (total) −0·12 (tumour)	0·023 (tumour)
Rat I......	10	0·37	0·037	15·0 (host)	0·47 (host)	0·031 (host)
				25·0 (total) − 1·5 (tumour)	1·10 (total) −0·04 (tumour)	0·026 (tumour)
Rat II ...	20	0·72	0·036	23·5 (host)	1·06 (host)	0·045 (host)
Rat III ...	20	0·64	0·032	10·0 (total) (No tumour)	0·85 (total) (No tumour)	0·085 (total) (No tumour)

up a given weight of tumour tissue than is necessary to build up an equal weight of somatic tissue. It is worth considering whether or not this result may, in itself, be an adequate explanation of the rapidity of growth of the tumour cells. The significance of this fact will be discussed in greater detail in a succeeding paper.

The question arises as to the source of the supply of nitrogenous material from which the tumour cells build up new tissue. We have seen already that in our experiments the tumour cells did not grow at the expense of the tissues of the host. They must therefore have elaborated the nitrogenous material taken in as food.

In a normal growing animal, part of the nitrogen of the food goes to repair the wear and tear which the cells have undergone; this fraction is represented by the nitrogen excretion of the animal in a state of nitrogen hunger. Another fraction is used for the building up of the growing tissues of the animal; this fraction is represented by the amount of nitrogen retained by the animal. A third fraction, which has no specific function and which can be replaced by fats or carbohydrates, serves as a source of energy; this fraction may vary within wide limits, and, together with the first-named fraction, it is represented by the amount of nitrogen excreted in the urine in an animal in a state of nitrogenous equilibrium. In the terminology of Rubner, these three fractions receive the name of "repair fraction" (Abnützungsquote), "growth fraction" (Wachstumsquote), and "ergogenic fraction" (dynamogener Verbrauch). It is obvious that a tumour growing in an animal does not derive its nitrogen from the repair fraction, since this fraction must remain constant if the animal is to live, and since it will be increased rather by the presence of the tumour, which throws an extra strain on the organism of the host. The tumour cells must therefore obtain their nitrogen either from the growth fraction or from the ergogenic fraction.

In the first case, we would have to assume that the cells of the tumour have a higher affinity for nutritive material than the growing cells of the host, and that they withdraw some of the nitrogenous material which otherwise should have gone to the building up of new tissues of the host. One would then expect the growth of the host to be retarded, and there should be a gradual diminution in the amount of nitrogen retained by the host.

If, on the other hand, the tumour cells derive their nitrogen from the ergogenic fraction, it would be unnecessary to assume any difference between the affinity of the tumour cells for nutritive material and that of the growing cells of the host. We would have to conceive that, after the wear and tear of the organism of the host is replaced the growing cells, be they those of the host or those of the tumour, have a first call upon the remainder of the nitrogen, and that only the nitrogen which remains after their demands have been satisfied goes to furnish energy. In other words, there would be a sparing of the protein undergoing combustion in the organism of the host. In this case one would expect to find the retention of nitrogen by the host to remain constant and its weight to increase, while the nitrogen excretion in the urine would gradually diminish.

Table IV gives the nitrogen retention *per diem* in three different periods. The first period of the nine days before transplantation represents the normal condition. The second period of the first nine days after transplantation

Table IV.

Nitrogen-retention of host during different periods.

Period.	Rat I.		Rat II.		Rat III.	
	Total in grammes.	Per diem in grammes.	Total in grammes.	Per diem in grammes.	Total in grammes.	Per diem in grammes.
Before transplantation (9 days)	0·37	0·04	0·72	0·08	0·64	0·07
After transplantation, 1st period (9 days)	0·22	0·02	0·61	0·07	0·51	0·055
2nd period (6 days)	Total ... 0·37 −Tumour 0·12 Host ... 0·25	— — 0·04	Total ... 0·47 −Tumour 0·04 Host ... 0·43	— — 0·07	0·35 No tumour	0·06

represents the condition where the absolute amount of tumour growth was small, and where therefore practically all the nitrogen retained must have gone to the host. During this period new stroma is formed and the tumour establishes itself. During the third period, starting from the tenth day to the sixteenth after transplantation, the absolute amount of tumour growth was large, and practically all the nitrogen found in the tumour when the animals were killed was retained during this period. If this amount is subtracted from the total amount of nitrogen retained during the third period, one arrives at the amount of nitrogen retained by the host during that period.

Any changes in the metabolism appearing in the first period after transplantation can be attributed mainly to the processes following upon inoculation (stroma-formation, etc.), while in the second period after transplantation those changes which are due to the growth of the tumour cells should come out most clearly. We find from Table IV that during the second period after transplantation the nitrogen retention by the host remains as high as it was before transplantation. Table I shows that the nitrogen excretion in the urine gradually diminishes as the tumour grows. In a former paper by one of us* it has been shown that the slow growth of a tumour may have a favourable influence on the growth of the host, and certainly does not retard it. From what has been said above, it is clear that in our experiments the tumour cells derived their supply of nitrogenous material by absorbing a part of that fraction of the nitrogen which otherwise would have served as a source of energy. They do not compete with the growing cells of the host, but they add their demands to those of the growing somatic cells.

It is obvious that as the tumour increases in size the amount of nitrogen necessary to replace the wear and tear of the cells of the tumour-bearing animal increases. At the same time a much larger amount of nitrogen will be necessary to cover the demands of the growing tumour cells, which rapidly increase in number. The result will be that the ergogenic fraction gets smaller and smaller and eventually a condition will arise when the host is incapable of absorbing sufficient nutritive material to cover the metabolic expenses of the host. The animal will then be in a state of under-feeding. The study of this condition will no doubt yield interesting results, but their correct interpretation will present great difficulties, since the essential features of the growth of cancer will be either masked or complicated by secondary factors. This condition will be discussed in another paper. We have referred to it here only in order to emphasise the fact that in our present investigation we have avoided these conditions, and that our present observations and conclusions are intentionally restricted to animals bearing tumours large enough to reveal any specific property or function of which the cells of a malignant new growth may be possessed, but not so large as to introduce secondary factors due merely to the excessive size of the tumours.

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* Cramer, "The Gaseous Metabolism of Rats inoculated with Malignant New Growths," 'Third Scientific Report of the Imperial Cancer Research Fund,' 1908, p. 427.

One other point must be noted, namely, the relation of the nitrogen retention to increase in weight of the somatic tissue of the host after transplantation (see Table III). In the case of the animal bearing a rapidly growing tumour (Rat I), this relation is the same after transplantation as it was before transplantation. In Rat III, however, when the initial proliferation had been followed by absorption, a remarkable change has taken place: the quotient is more than twice as high after transplantation as it was before transplantation. In other words, nitrogen had been retained out of proportion to the increase in weight. It might be suggested that some of the tissues of this animal had been so changed in composition that they contained more nitrogen after transplantation. This explanation is not a very plausible one, and the analysis of the various tissues, which will be given in the succeeding paper, show that it cannot be maintained, since the nitrogen percentage of the various tissues of Rat III agrees with the nitrogen percentage of the tissues of Rat I and Rat II.

It is only possible to explain this phenomenon by the assumption that a formation of nitrogenous tissue has taken place, while at the same time non-nitrogenous tissue (fat or glycogen) has been used up. Whether this process stands in any relation to the absorption of tumour tissue and the effects produced by such an absorption (immunisation), will have to be determined by further investigations. The marked constitutional changes which accompany and follow the absorption of a tumour leave little doubt that the metabolism of the animal is deeply affected by this process. It is suggestive to find that in Rat II, where there was apparently a concomitant absorption of the tumour, a slight increase in the quotient is found.

Summary.

The main result of these experiments is to be found in the following facts:—

1. Less nitrogen is necessary to build up a certain weight of tumour tissue than is necessary to build up an equal weight of the somatic tissues of the host.

2. Animals bearing tumours maintain their positive nitrogen balance, and the nitrogen retention actually increases with the size of the tumour.

3. In our experiments the cells of the new growth derived their nitrogenous material necessary for the building up of new tissue by a sparing action on

the protein metabolism. The tumour cells do not proliferate at the expense of the tissues of the host, nor is there any evidence that they have a higher affinity for nutritive material than the growing cells of the host.

4. There is no evidence of the existence of substances secreted by the tumour disturbing the nitrogenous metabolism by means of a toxic action on the tissues of the host.

5. It is specially pointed out that these conclusions refer only to animals bearing tumours of sufficient size to warrant the assumption that they would reveal any specific property or function which may be possessed by the cells of a neoplasm. The effects which a large tumour must necessarily produce by virtue of its mere mass are not here considered.

The expenses of this research have been defrayed by grants from the Moray Research Fund of the University of Edinburgh.

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*Contributions to the Biochemistry of Growth.**—*Distribution of Nitrogenous Substances in Tumour and Somatic Tissues.*

By W. Cramer and Harold Pringle.

(Communicated by Prof. E. A. Schäfer, F.R.S. Received February 3,—
Read February 24, 1910.)

(From the Physiology Department, University of Edinburgh, and the Imperial Cancer Research Fund, London.)

In the preceding paper† a determination of the distribution of the nitrogen retained during a metabolism experiment showed that less nitrogen is needed to build up a given weight of tumour tissue than is necessary to build up an equal weight of the somatic tissues of the host. If this result is correct, it would follow that cancerous tissue should have a lower nitrogen percentage than the somatic tissues of the host.

We have therefore carried out nitrogen estimations of various tissues of Rats I, II, and III used in the experiments described in the preceding paper.

In order to make our results applicable to carcinomatous tumours, we examined the tissues of mice of about the same age, bearing a rapidly growing adeno-carcinoma (Tumour B obtained from the Imperial Cancer Research Fund). Our results are given in Table I.

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* This research is in continuation of papers in 'Roy. Soc. Proc.,' B, vol. 80, 1908, p. 263, and this vol., p. 307, *supra*.

† W. Cramer and Harold Pringle, 'Roy. Soc. Proc.,' *supra*, p. 307.

Table I.—Giving the Absolute Amounts of Total Nitrogen expressed in Percentage of Weight of Tissue, in the Various Tissues.

Animals.	Tumour.		Heart.		Muscle.		Liver.		Kidneys.	
	Amount of tissue used in grammes.	N percentage.	Amount of tissue used in grammes.	N percentage.	Amount of tissue used in grammes.	N percentage.	Amount of tissue used in grammes.	N percentage.	Amount of tissue used in grammes.	N percentage.
Rat I	1·095 1·025	2·31 2·47	0·8040	3·04	0·3400	3·74	1·0310	3·04		
Rat II	0·2970 0·3230	2·83 2·77	0·667	3·23	0·4900	3·63	0·5540	3·26		
Rat III	No tu	mour	0·8400	3·06	0·3850	3·49	0·5720	3·08		
Mouse I ...	0·4134 0·4390	2·27 2·23			0·3816 0·4262	2·86 2·69	0·2900 0·3170	3·14 3·09	0·1780	2·98
Mouse II ...	0·5898 0·7444	2·25 2·33					0·2946 0·3240	2·75 2·80	0·2426	3·01
Necrotic part of same tumour	0·3193 0·3218	2·37 2·39								
Mouse III ...	0·7432 0·7700	2·29 2·29			0·3073 0·2435	2·94 2·82	0·3946 0·3686	3·19 3·19	0·1869	3·29

It will be seen that the amount of total N for the same tissues is remarkably constant for different animals of the same species. In every case the tumour tissue contains less nitrogen than the somatic tissues of the host. This is true both for the sarcomatous and for the carcinomatous tumours. In order to get an idea of the distribution of the nitrogenous substances, we have divided them into (*a*) those which are coagulated by boiling alcohol, and (*b*) those which are not coagulated. The latter do not give the biuret test. These two groups may be taken to correspond roughly with native proteins on the one hand and simpler abiuret substances on the other.

The determination of the relative amounts of coagulable and incoagulable nitrogenous material was carried out by the method which we have used in previous investigations.* This method we have found to give uniform and reliable results, and to be well adapted for the purpose of determining the distribution of coagulable protein material in the various tissues. For this investigation we used the tissues of Rats I, II, and III, as well as those of a normal rat and of two rats, X and Y, of about the same weight, bearing very large tumours also derived from the same transplantable rat sarcoma (J. R. S.). The tumours of Rats X and Y were so large (30 grammes and 54 grammes respectively), that they had begun to grow at the expense of the tissues of the host. Rat Y was emaciated when killed. We also analysed the tissues of two mice which had carcinomatous tumours of medium size. The amount of tissue available from these latter being small, the tissues from the two were pooled and analysed together, and so the figures are the average for two. All the animals were kept on the same diet, viz., bread and milk.

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* Harold Pringle and W. Cramer, 'Journal of Physiology,' vol. 37, p. 2, 1908.

The following table gives the result of the analyses of various tissues, the amounts of coagulable and incoagulable N are given in percentage of total N, and the figures indicate therefore the relative proportions in which the two groups of substances referred to above occur in the various tissues.

Table II.—Giving Proportion of Coagulable and Incoagulable Nitrogen expressed in Percentage of Total Nitrogen present in the Various Tissues.

Animal.	Tumour.		Kidney.		Liver.		Muscle.	
	Incoag. N percentage.	Coag. N percentage.	Incoag. N percentage.	Coag. N percentage.	Incoag. N percentage.	Coag. N percentage.	Incoag. N percentage.	Coag. N percentage.
Rat I	19·73	80·27	13·80	86·20	9·90	90·10		
Rat II	20·64	79·36	11·85	88·15	9·90	90·10		
Rat III	No tu	mour	14·10	85·90	10·40	89·60		
Rat X	15·70	84·30	17·97	82·03	13·83	86·17		
Rat Y	18·74	81·26	14·37	85·63	14·20	85·80		
Normal Rat Z ...	No tu	mour	14·37	85·63	11·60	88·40		
2 mice (carcinoma)	23·00	77·00	—	—	17·50	82·50	16·00	84·00

Referring to Table II, it will be seen that the analyses of the tissues of Rats I and II which had small tumours agree together; and that the analyses of the somatic tissues of these also agree with those of Rats III and Z, which were normal animals. Rats X and Y, which had large tumours, begin to show anomalies, particularly the kidney of Rat X, where the proportion of coagulable to incoagulable nitrogen deviates markedly from the normal.

There is a marked difference in the relative amount of coagulable nitrogen in the different tissues examined, and it will be seen that, with the exception of Rat X, the tumour tissue in every case had a considerably smaller percentage of coagulable nitrogen than the somatic tissues, liver, kidney, or muscle (in the case of the mice).

The average figures for the percentage of coagulable nitrogen are as follows:—

	Tumour.	Kidney.	Liver.	Muscle.
Rats with tumours	81·3	85·50	88·04	
Rats, normal	—	85·76	89·0	
Mice with tumours......	77·0	—	82·5	84·0

It will be seen that the proportion between coagulable and incoagulable nitrogen in tumour tissue and in somatic tissue is different in such a way that in the case of the rats the relative amount of the coagulable nitrogen of the tumour tissue is 4·2 per cent. less than in kidney, and 6·7 per cent. less than in liver. In the case of the mice it is 5·5 per cent. less than in the liver and 7 per cent. less than in muscle.

In order to determine the absolute amounts of coagulable and incoagulable nitrogen it is necessary to correlate these figures with the absolute amounts of total nitrogen in the liver and the tumour* which are available in the case of Rats I, II, and III from the protocol of the preceding paper.† In the case of Rats X and Y, no determinations of the absolute nitrogen-content of the various tissues were made, and as the stage of tumour growth was different in the two groups, we have thought it better not to make the results obtained from Rats I and II applicable to Rats X and Y. In the case of the mice, the small quantity of tissue available made it impossible to make estimations of absolute amount of nitrogen and also of the coagulable and incoagulable fractions in the same animal. We have therefore calculated our results in the case of the mice by correlating our estimations of the total nitrogen given in Table I with the figures giving the relative quantities of coagulable and incoagulable nitrogen as given in Table II.

Table III gives the absolute amount of coagulable and incoagulable nitrogen calculated from our data as above.

The agreement of the figures obtained for the absolute amounts of coagulable and incoagulable nitrogen in the somatic tissues obtained in the series of Rats I, II, and III by individual estimations is striking, and affords

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* It was impossible to carry out duplicate determinations of total nitrogen, coagulable and incoagulable nitrogen, in the same animal in the case of other tissues owing to the small amount available.

† W. Cramer and Harold Pringle, 'Roy. Soc. Proc.,' *supra*, p. 307.

Table III.—Giving the Absolute Amounts of Total Nitrogen, Coagulable Nitrogen, and Incoagulable Nitrogen, expressed in Percentage of the Weight of Tissue, in the Various Tissues.

Animal.	Tumour.			Liver.			Muscle.		
	Total N.	Coag. N.	Incoag. N.	Total N.	Coag. N.	Incoag. N.	Total N.	Coag. N.	Incoag. N.
Rat I	2·39	1·92	0·47	3·04	2·74	0·30			
Rat II	2·80	2·22	0·58	3·26	2·94	0·32			
Rat III	No	tumour		3·08	2·76	0·32			
Mouse I ...	2·25 } 2·28	1·76	0·52	3·12 } 3·03	2·50	0·53	2·77 } 2·83	2·38	0·45
Mouse II ...	2·29			2·77			—		
Mouse III ...	2·29			3·19			2·88		

evidence of the reliability of the methods. The table shows that both for mice and rats, and for carcinomata and sarcomata, the tissues of rapidly proliferating malignant new growths show a marked diminution, amounting to about one-quarter, of the substances which can be coagulated by alcohol. The substances which are not coagulated by alcohol show a slight but distinct increase as compared with somatic tissue.

Summary.

The rapidly growing cells of a malignant new growth, and the cells of the animal bearing it, show a marked quantitative difference in their chemical composition. Weight for weight, the cancer cells contain only about three-fourths of the protein substances present in the tissues of the host. In other words, with the same amount of protein a bigger mass of tumour tissue than of host tissue can be built up. The simpler (abiuret) nitrogenous products of cell metabolism, however, are present in slightly greater amount in the cancerous tissue.

These results are important in themselves, for the light they throw upon the chemistry and the metabolism of the cancer cell. The interpretation of their bearing on the growth of cancerous tissue may only be attempted with caution. As regards the rapidity of growth, it is possible to formulate conclusions; since the tissue of a neoplasm can be built up with less protein than the same weight of host tissue, the former must grow more rapidly than the latter under circumstances where both are using up nitrogen for mere growth at the same rate. In order to explain the rapidity of growth, it is not necessary to assume that the cancer cells build up protein more rapidly in a given time than the cells of the host, since we have shown that the former require less protein, and in the preceding paper we have been unable to find any evidence in favour of the assumption that tumour cells have a higher affinity for the material necessary for the building up of new tissue.

If the significance we attach to the relation between the diminished nitrogen-content and the rapidity of growth of cancerous tissue is justified, the same relation should hold good not only for the tissues of a malignant new growth but equally well for any other rapidly growing tissue. In a former paper* we have pointed out the similarity which exists between the growth of cancer and the growth of the fœtus, and, in fact, preliminary experiments by Dr. J. Lochhead have shown that the tissues of the fœtus have a lower nitrogen percentage than those of the maternal organism.

The observations are being extended to a series of transplantable tumours of all grades of rapidity of growth and varied degrees of histological differentiation.

The expenses of this research have been defrayed by a grant from the Moray Research Fund of the University of Edinburgh.

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* Cramer, "The Gaseous Metabolism in Rats inoculated with Malignant New Growths," 'Third Scientific Report of the Imperial Cancer Research Fund,' 1908, p. 427.

The Alcoholic Ferment of Yeast-juice. Part V.—*The Function of Phosphates in Alcoholic Fermentation.*

By ARTHUR HARDEN, F.R.S., and WILLIAM JOHN YOUNG (Biochemical Laboratory of the Lister Institute of Preventive Medicine).

(Received February 12,—Read February 24, 1910.)

Two equations have been proposed by the authors* to represent the course of alcoholic fermentation by yeast-juice:

(1) $2C_6H_{12}O_6 + 2PO_4HR_2 = 2CO_2 + 2C_2H_6O + 2H_2O + C_6H_{10}O_4(PO_4R_2)_2$,
(2) $C_6H_{10}O_4(PO_4R_2)_2 + 2H_2O = C_6H_{12}O_6 + 2PO_4HR_2$.

These were founded on (*a*) the determination of the amount of carbon dioxide and alcohol produced by the addition of a known amount of phosphate in presence of excess of sugar†; (*b*) the production of a hexosephosphate of the composition $C_6H_{10}O_4$ $(PO_4R_2)_2$; (*c*) the occurrence of an enzymic hydrolysis of this substance with production of free phosphate.

In order to obtain further experimental justification for this view, several additional determinations have been made, and these form the subject of the present paper.

I. *Ratio of Carbon Dioxide produced to Sugar fermented in Presence of Excess of Phosphate.*

When glucose or fructose is fermented by yeast-juice in presence of excess of phosphate, a period of accelerated fermentation occurs, during which two molecules of carbon dioxide are evolved and one molecule of hexosephosphate formed from two molecules of sugar added. The ratio between sugar added and carbon dioxide evolved is determined by adding a known weight of sugar, together with an excess of sodium phosphate to yeast-juice at 25°. The phenomena then observed are precisely similar to those which occur when a phosphate is added to a fermenting mixture of yeast-juice and excess of sugar, as previously described (*loc. cit.*). The rate of fermentation rapidly rises, and then gradually falls until a rate is attained approximately equal to that of the auto-fermentation of the juice in presence of phosphate. At this point it is found that the extra carbon dioxide evolved, beyond the amount which would have been given off in the absence of added sugar, is equivalent to only half the sugar according to the equation:

$$C_6H_{12}O_6 = 2CO_2 + 2C_2H_6O,$$

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* 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 299.

† 'Roy. Soc. Proc.,' B, 1906, vol. 77, p. 405.

but exactly equivalent to the sugar according to the authors' equation (1) as given above.

Experiments 1 *and* 2.—Two quantities of 25 c.c. of yeast-juice were incubated at 25° for 15 minutes.

1. To one was added 4 c.c. of a 0·6 molar solution of dipotassium hydrogen phosphate, saturated with carbon dioxide, and 1 c.c. of a solution containing 0·2 gramme of fructose (pure from inulin). The volumes of gas evolved in successive periods of 5 minutes were then read. As soon as a steady rate had been attained, a second addition of 4 c.c. of potassium phosphate solution and 1 c.c. of fructose solution was made, and the volumes of gas evolved observed as before.

2. The second portion of yeast-juice was treated in the same way, except that 0·2 gramme of pure glucose was substituted for the fructose.

The following are the actual readings:—

Time after addition.	Fructose.		Glucose.	
	First addition.	Second addition.	First addition.	Second addition.
	c.c.	c.c.	c.c.	c.c.
5	20·6	20·5	6·9	17·0
10	9·2	8·2	9·7	
15	4·2	4·7	12·0	12·8
20	2·8	3·6	6·2	5·4
25	2·4	2·6	3·0	3·3
30	1·9	2·6	2·3	2·6
35	2·2	2·1	1·8	2·2
Total	43·3	44·3	41·9	43·3
Fermentation of juice at 2·2 c.c. per 5 mins.	15·4	15·4	15·4	15·4
Extra carbon dioxide ...	27·9	28·9	26·5	27·9

The gas was measured moist at 17° and 762·4 mm.

The volume calculated at the same temperature and pressure—

(*a*) From the ratio $C_6H_{12}O_6 : 2CO_2 = 53·92$

(*b*) „ $2C_6H_{12}O_6 : 2CO_2 = 26·96$.

II. *Fermentation in Presence of Small Amounts of Phosphate.*

Numerous attempts have been made to procure conclusive experimental evidence that alcoholic fermentation cannot take place in the absence of phosphates. Hitherto, however, it has not been found possible to free the materials employed completely from phosphorus compounds which yield phosphates by enzymic hydrolysis during the experiment, but it has been found that when the phosphate contents are reduced to as low a limit as possible, the amount of sugar fermented becomes correspondingly small, and, further, that in these circumstances the addition of a small amount of phosphate produces a relatively large increase in the fermenting power of the enzyme.

It was previously shown that the addition of phosphate to a mixture of yeast-juice and a sugar not only caused the evolution of an equivalent amount of carbon dioxide, but, in addition, produced an increase in the total fermentation amounting to from 10 to 150 per cent. of the original.

It now appears that when the total phosphorus present is largely reduced, the increase produced by the addition of a small amount of phosphate may amount to as much as 700 per cent. of the original, in addition to the quantity equivalent to the phosphate, whilst the actual total evolved, including this equivalent, may be as much as twenty times the original fermentation. This result must be regarded as strong evidence in favour of the view that phosphates are indispensable for alcoholic fermentation.

The results indicated above were experimentally obtained in three different ways. In the first place (Experiments 3 and 4) advantage was taken of the fact that the residues obtained by filtering yeast-juice through a Martin gelatin filter are sometimes found to be almost free from mineral phosphates, whilst they still contain a small amount of coferment. The experiment then consists in comparing the fermentation produced by such a residue poor in phosphate with that observed when a small amount of phosphate is added. The second method (Experiment 5) consisted in carrying out two parallel fermentations by means of a residue rendered inactive by filtration and a solution of coferment free from phosphate and hexosephosphate (prepared by a method shortly to be described) and adding a small amount of phosphate to one of the solutions.

The third method (Experiment 6) consisted in washing zymin with water to remove soluble phosphates and then adding to it a solution of coferment containing only a small amount of phosphate, and ascertaining the effect upon the fermentation of the addition of a small known amount of phosphate.

Experiment 3.—A slightly active filtration-residue was employed, 1 gramme of which contained a total amount of phosphorus corresponding with 0·038 gramme of $Mg_2P_2O_7$.

a. 1 gramme residue + 2 grammes fructose + 20 c.c. water.
b. The same mixture + 0·26 gramme crystallised sodium phosphate.

Experiment 4.—A second slightly active residue was used, the total phosphorus in which was not estimated.

a. 1 gramme residue + 2 grammes fructose + 20 c.c. water.
b. The same mixture + 0·25 gramme crystallised sodium phosphate.

Experiment 5.—In this case an inactive filtration-residue containing no free phosphate and total phosphorus equivalent to 0·0342 gramme $Mg_2P_2O_7$, and a solution of coferment free from mineral phosphate and hexosephosphate, but containing total phosphorus equivalent to 0·077 gramme $Mg_2P_2O_7$ per 25 c.c., were used.

a. 1 gramme residue + 2 grammes fructose + 25 c.c. coferment solution.

b. The same + 0·25 gramme crystallised sodium phosphate.

Experiment 6.—*a.* 25 c.c. washed zymin suspension + 15 c.c. coferment solution + 2 grammes fructose.

b. The same mixture + 2·5 c.c. of a 0·3 molar solution of sodium phosphate solution.

All the experiments were carried out at 25°, in presence of toluene, and the results are tabulated below.

	Experiment			
	3.	4.	5.	6.
	c.c.	c.c.	c.c.	c.c.
Gas evolved in absence of added phosphate ...	1·4	1·2	20·3	31·2
Gas evolved in presence of added phosphate ...	25·8	26·8	92·3	187·2
Increase due to phosphate	24·4	25·6	72·0	156
Carbonic acid equivalent to phosphate............	16·9	16·8	16·8	19
Increase after initial period............	7·5	8·8	55·2	137
Ratio of totals	18·4	21·3	4·5	6
Ratio of increase after initial period to original fermentation	5·3	7·3	2·7	4·4

III. *Hydrolysis of Hexosephosphate by Yeast-juice with Production of Free Phosphate and a Fermentable Sugar.*

(*a*) Production of free phosphate and a reducing substance.

It has previously been shown that free phosphate is produced by the action of the enzymes of yeast-juice on the hexosephosphate formed during fermentation.* According to equation (2) above, this phosphate should be accompanied by a sugar, and the following experiment shows that this is actually the case :—

Experiment 7.—Potassium hexosephosphate was incubated with an inactive filtration-residue, so that no fermentation of any liberated hexose could occur, and the amounts of free phosphate and reducing substance formed were determined, allowance being made for the quantities produced by the incubation of the residue and of the hexosephosphate separately.

5 grammes of an inactive filtration-residue were dissolved in water and made to 75 c.c., and a solution of potassium hexosephosphate, free from glucose and free phosphate, and

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* Harden and Young, 'Roy. Soc. Proc.,' B, 1908, vol. 80, p. 299.

containing phosphorus equivalent to 0·2352 gramme $Mg_2P_2O_7$ in 25 c.c., was prepared. The following mixtures were then made and incubated at 25° in presence of toluene :—

(1) 25 c.c. residue + 25 c.c. water.
(2) 25 c.c. hexosephosphate + 25 c.c. water.
(3) 25 c.c. residue + 25 c.c. hexosephosphate.

After 5 hours' incubation the solutions were heated for 5 minutes in boiling water, made up to a known volume, and the phosphate and reducing power estimated in aliquot portions of the filtrate. In order to ascertain the reducing power of the product, it was necessary to remove the unaltered hexosephosphate, and this was effected by precipitation with basic lead acetate, the estimation of the reducing power being made by means of Pavy's ammoniacal copper solution. In the following table the amounts of phosphate are expressed as grammes of $Mg_2P_2O_7$, and the reducing power as grammes of glucose in the quantities originally incubated.

Solutions.	Free phosphate as $Mg_2P_2O_7$.			Reducing substance as glucose.		
	Before.	After.	Produced.	Before.	After.	Produced.
1	0	0·0141	0·0141	0	0	0
2	0·0009	0·0030	0·0021	0·0244	0·0275	0·0031
3	0·0009	0·0968	0·0959	0·0244	0·0595	0·0351

The amount of reducing substance expressed as glucose produced by enzymic hydrolysis is therefore 0·0351 − 0·0031 = 0·032, and that of phosphate is 0·0959 − (0·0141 + 0·0021) = 0·0818 gramme.

(*b*) Proof that the reducing substance is a sugar.

Experiment 8.—50 grammes of zymin (Schroder) were washed in the centrifuge four times with water, in order to remove as much as possible of the coferment and thus to diminish the fermenting power of the zymin, and the residue was made to 120 c.c. with water. The following solutions were then incubated, Nos. 2 and 3 being added to ascertain the amount of sugar formed by the spontaneous hydrolysis of the hexosephosphate and from the carbohydrates of the zymin :—

1. 50 c.c. washed zymin suspension + 55 c.c. of a solution of sodium hexosephosphate, equal to 19·9 c.c. normal solution, and equivalent if completely fermented to 223 c.c. of carbon dioxide at N.T.P.
2. 50 c.c. washed zymin suspension + 55 c.c. water.
3. 50 c.c. water + 55 c.c. sodium hexosephosphate solution.

No. 1 gave a total evolution of 25·3 c.c. carbon dioxide under atmospheric conditions, whilst Nos. 2 and 3 gave no gas. At the end of 48 hours all three solutions were removed, filtered, and precipitated with lead acetate in neutral solution in order to remove all phosphate and unchanged hexosephosphate. The excess of lead was then precipitated in the filtrates with sulphuretted hydrogen, and the filtrates, after removal of the sulphuretted hydrogen by a current of air, were neutralised and all made up to the same volume.

The following reactions for the sugars were then tried with equal volumes of each solution :—

	1.	2.	3.
a. Fehling's solution	Reduction	Slight reduction	No reduction
b. Mohlisch's *α*-naphthol reaction	Intense violet coloration	Violet coloration	No reaction
c. Seliwanoff's resorcinol reaction for fructose	Fair reaction	Very faint reaction	No reaction

50 c.c. of each solution were mixed with equal quantities of phenylhydrazine dissolved in acetic acid, and heated in boiling water for 1 hour. Osazones were formed in Solutions 1 and 2, but not in 3. These osazones were filtered off, washed with water, dried, washed with ether, again dried and weighed. That from No. 1 weighed 0·09 gramme; that from No. 2, 0·01 gramme. No. 1 melted at 166°—168° and after recrystallisation from boiling toluene, at 185°—186°. The osazone from No. 2 melted at 196°—198°.

Hence a sugar has been produced by the action of zymin freed from coferment on the hexosephosphate, which has the characteristic reactions of fructose, although the presence of other hexoses is not excluded.

A sugar of the same properties has already been shown to be formed when a hexosephosphate is hydrolysed by acids.*

The decomposition products of hexosephosphate by enzymic and acid hydrolysis appear, therefore, to be the same.

(*c*) Fermentation by yeast of the sugar produced by the enzymic hydrolysis of hexosephosphate.

Experiment 9.—20 grammes of zymin (Schroder) were washed as before and made to 100 c.c. with water. The following solutions were then incubated at 25° in presence of toluene :—

1. 50 c.c. of this suspension + 40 c.c. of a solution of sodium hexosephosphate, equal to 27 c.c. of normal solution, and equivalent, when completely fermented, to 302 c.c. of carbon dioxide at N.T.P.

2. 50 c.c. zymin suspension + 40 c.c. water.

3. 50 c.c. water + 40 c.c. sodium hexosephosphate solution.

No. 1 gave a total evolution of 167 c.c. of carbon dioxide, whilst Nos. 2 and 3 gave no gas. At the end of three days the solutions were filtered, and treated as in Experiment 8. The final solutions, free from hexosephosphate, were then incubated with a suspension of well-washed living yeast, which had previously been autofermented for some days.

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* Young, 'Roy. Soc. Proc.,' B, 1909, vol. 81, p. 528.

	Gas evolved.	Gas evolved, corrected for auto-fermentation.
	c.c.	
a. 15 c.c. yeast suspension + 46 c.c. No. 1	84·1	77·5
b. 15 " " + 46 " No. 2	36·4	29·8
c. 15 " " + 46 " No. 3	9·4	2·8
d. 15 " " + 46 " water	6·6	

The carbon dioxide derived from the sugar produced by the enzymic hydrolysis of the hexosephosphate by the zymin is therefore equal to $77·5-(29·8+2·8) = 44·9$ c.c.

Hence the sugar formed by digesting hexosephosphate with zymin is readily fermentable by yeast.

(*d*) Production of carbon dioxide from hexosephosphate by yeast-juice and zymin.

It follows from the facts recounted above, which are expressed in equation (2) at the commencement of the paper, that the products of the enzymic hydrolysis of the hexosephosphates should be fermented by yeast-juice. Since, moreover, the enzyme producing this hydrolysis, which may be termed *hexosephosphatase*, is present in yeast-juice, the hexosephosphate itself when added to yeast-juice should undergo gradual hydrolysis and fermentation. The whole of the phosphorus would then be liberated as free phosphate, and the carbohydrate residue would be converted into alcohol and carbon dioxide.

The following experiments show that hexosephosphate is actually fermented by yeast-juice with evolution of carbon dioxide and production of free phosphate:—

Experiment 10.—Two quantities of 25 c.c. of yeast-juice were incubated at 25° in presence of toluene until a steady rate of auto-fermentation had been attained. (*a*) To one were added 15 c.c. of water; (*b*) To the other were added 15 c.c. of a solution of sodium hexosephosphate containing phosphorus equivalent to 0·4673 gramme of $Mg_2P_2O_7$ and therefore capable of yielding 94·3 c.c. of carbon dioxide.

	CO_2 evolved in 40 hours.
	c.c.
a.	85
b.	131·8
Difference......	46·8 at 19° and 762 mm. = 42·97 c.c. at N.T.P.

Somewhat less than half the available carbon dioxide was therefore evolved.

In this case the auto-fermentation of the yeast-juice was a considerable fraction of the total fermentation produced in presence of the hexosephosphate. This introduces an uncertainty, as the additional phosphate constantly being formed in (*b*) might accelerate the auto-fermentation to such an extent as greatly to increase the total evolved. In order to avoid this complication as far as possible, a second experiment was carried out with yeast-juice which was prepared from yeast previously kept in the pressed condition at 20° for 20 hours, the juice being then treated with living yeast for three hours before use in order to ferment away any free sugar. In this way a juice of small auto-fermenting power was obtained. Unfortunately the fermenting power of the juice towards sugar was also considerably lowered by this treatment.

Experiment 11.—Yeast-juice, treated as described above, was employed, and two quantities were taken—

a. 30 c.c. of the juice + 30 c.c. of a solution of potassium hexosephosphate, equivalent to 134·6 c.c. of carbon dioxide.

b. 30 c.c. of the juice + 30 c.c. of water.

These mixtures were incubated in presence of toluene, and the gas evolved and phosphate formed were estimated.

	Carbon dioxide.		Phosphate rendered free, as $Mg_2P_2O_7$.
	c.c.	gramme.	
a …………	52·3	0·0944	0·2058
b …………	14·5	0·0262	0·0792
Difference ……	37·8	0·0682	0·1266

Here the fermentation of the hexosephosphate has yielded at least 0·0682 gramme of carbon dioxide and 0·1266 gramme of phosphate as $Mg_2P_2O_7$.

The fact that hexosephosphate is fermented by zymin has been observed by Iwanoff,* and this observation has been confirmed by the authors.

IV. *Nature of the Chemical Change involved in Alcoholic Fermentation.*

The facts (1) that alcoholic fermentation occurs according to the equation:

$$2C_6H_{12}O_6 + 2PO_4HR_2 = 2CO_2 + 2C_2H_6O + 2H_2O + C_6H_{10}O_4(PO_4R_2)_2,$$

and (2) that the hexosephosphates produced from glucose, fructose, and mannose appear to be identical† have an important bearing on the chemical interpretation of the decomposition by fermentation of the hexoses into carbon dioxide and alcohol. It has been pointed out by Young (*loc. cit.*) that the identity of the hexosephosphates from these three sugars may possibly be explained in either of two ways. In the first place it is to be noted that these three sugars have a common enolic form, and the hexosephosphate may be a derivative of this. In the second place, it is possible that the two molecules of sugar which are involved in the reaction, of which the equation is given above, may be decomposed into smaller groups, and that the hexosephosphate may be formed by a synthesis from these. As the formation of the hexosephosphate is invariably accompanied by that of an equivalent amount of carbon dioxide and alcohol, the second explanation appears the more probable, as it provides a source for the simultaneous production of these substances.

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* 'Centr. Bakt.,' Abt. II, 1909, vol. 24, p. 1.

† Young, 'Roy. Soc. Proc.,' B, 1909, vol. 81, p. 528.

According to this view two molecules of the hexose, or possibly of the enolic form, are each decomposed primarily into two groups. Of the four groups thus produced, two go to form alcohol and carbon dioxide, and the other two are synthesised to a new chain of six carbon atoms, which forms the carbohydrate residue of the hexosephosphate. The introduction of the phosphoric acid groups may possibly occur before the rupture of the original molecules, and may even be the determining factor of this rupture, or, again, this introduction may take place during or after the formation of the new carbon chain. Sufficient information is not yet available for the exact formulation of a scheme for this reaction. Such a scheme, it may be noted, would not necessarily be inconsistent with the views of Wohl and Buchner as to the way in which the carbon chain of a hexose is broken in the process of fermentation, but would interpret differently the subsequent changes which are undergone by the simpler groups which are the result of this rupture.

It may, however, be pointed out that if the hexosephosphate be a derivative of the enolic form common to mannose, glucose, and fructose, the compound derived from galactose should be different. If, on the other hand, the hexosephosphate be derived from fractions of two hexose groups, the galactose compound might, or might not, be different from that derived from the other hexoses. The enolic form of galactose differs from that of mannose, glucose, and fructose, in the symmetry of its γ-carbon atom, and the production of a hexosephosphate different from, or identical with, that derived from glucose, would depend on the preservation or destruction of the symmetry of this carbon atom in the reaction. It appears, therefore, that a study of the behaviour of galactose towards phosphate in the presence of the yeast enzymes may have an important bearing on the question, and experiments on this subject are now in progress.

Summary.

1. When glucose or fructose is added to yeast-juice in presence of excess of phosphate, a period of accelerated fermentation occurs, during which the added sugar undergoes the reaction:

$$2C_6H_{12}O_6 + 2PO_4HR_2 = 2CO_2 + 2C_2H_6O + 2H_2O + C_6H_{10}O_4(PO_4R_2)_2,$$

one molecule of carbon dioxide being evolved for each molecule of sugar added.

2. When the available phosphate of a mixture of ferment, coferment, and sugar is greatly reduced, the total fermentation produced becomes very small. The addition of a small amount of a phosphate to such a mixture produces a relatively large increase in the total fermentation, even after allowing for the amount of carbon dioxide equivalent to the phosphate added.

3. A hexosephosphate when digested with yeast-juice is hydrolysed by an enzyme, hexosephosphatase, with production of free phosphate and a sugar which is capable of being fermented by yeast.

As the result of this hydrolytic action, the hexosephosphates, when treated with yeast-juice or zymin, are finally converted into carbon dioxide, alcohol, and free phosphate.

4. In discussing the chemical changes which the molecule of sugar may undergo in the process of fermentation, it is necessary to take into consideration the fact that two molecules of sugar are involved in the reaction.

The Causes of Absorption of Oxygen by the Lungs (Preliminary Communication).

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(Received February 22,—Read March 10, 1910.)

It has for long been a subject of controversy among physiologists whether the absorption of oxygen through the walls of the lung alveoli into the blood occurs simply by diffusion or by an active physiological process analogous to glandular secretion.* Pflüger, Fredericq, and, quite recently, Krogh,† have brought forward experimental evidence in favour of the diffusion theory, while Bohr, and Haldane and Lorrain Smith have supported the secretory theory, which was originally suggested by Ludwig.

If the partial pressure of oxygen is ever greater in the arterial blood leaving the lungs than in the air of the lung alveoli, it is clear that the simple diffusion theory must fall to the ground; and the experiments hitherto made have been designed to ascertain whether or not the oxygen pressure in the arterial blood is ever greater than in the alveolar air. Bohr, Fredericq, and Krogh have with this object employed improved forms of Pflüger's well-known "aerotonometer," the instrument finally devised by Krogh being very perfect of its kind. Haldane and Lorrain Smith's method depends upon the following facts:—When blood is brought into prolonged and intimate contact with a mixture of carbon monoxide and air the hæmoglobin of the blood-corpuscles combines partly with the oxygen and partly with the carbon monoxide, the final proportions (which can be easily and accurately determined) depending, in accordance with the laws of mass-action, on the relative partial pressures of the oxygen and carbon monoxide, and on a constant. Hence, if the final proportions, the constant, and the partial pressure of the carbon monoxide are known, the partial pressure of the oxygen can be accurately deduced. By supplying to an animal air containing a fixed proportion of carbon monoxide, until the final saturation of its hæmoglobin with carbon monoxide is reached, the partial pressure of oxygen in its arterial blood can be calculated on the same principle. If the arterial oxygen pressure is greater than that of the alveolar air the final saturation of the hæmoglobin with carbon monoxide will be less than that of blood saturated with the same air outside the body, and *vice versâ.* It is, of course, assumed that carbon monoxide diffuses freely through the body, since, apart from its property of combining with the hæmoglobin, it is, as was experimentally shown by Haldane, a physiologically indifferent gas, like nitrogen or hydrogen.

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* A comprehensive account of this controversy is given by Bohr in Nagel's 'Handbuch der Physiologie,' vol. 1, p. 142, 1905.

† 'Skand. Archiv für Physiologie,' vol. 23, pp. 179—278, 1910.

Haldane and Lorrain Smith determined the constants required in their calculations, not from experiments at body temperature on blood from the animals experimented on, but on dilute blood solutions or undiluted human blood. This omission, which in the then existing state of knowledge did not seem important, has, we find, seriously affected their estimates of the oxygen pressure in the arterial blood. The constant varies distinctly for the blood of different animals, and even for different individuals of the same species. We have, therefore, repeated the experiments, using mice, on which most of Haldane and Lorrain Smith's experiments were made, and determining the constant for the blood of each animal experimented on. We find that in general their estimates of oxygen tension were about a third too high.

We have also supplied an omission in their experiments by investigating the arterial oxygen pressure in animals breathing a very low percentage of carbon monoxide (less than 0·02 per cent.), so that no physiological disturbance is produced by the gas.

Our results with mice are as follows:—

1. When a very low percentage of carbon-monoxide is breathed, so that no want of oxygen is produced in the body, the arterial oxygen pressure is slightly *below* the alveolar oxygen pressure. Apparently, therefore, the absorption of oxygen is by diffusion alone, as indicated by the experiments of Fredericq and Krogh, and by most of Bohr's experiments. The results agree closely with Bohr's recent calculations of the arterial oxygen pressure which might be expected during rest if diffusion alone were in play.

2. When a much higher percentage of CO is breathed (0·2 per cent. or more) the oxygen pressure in the arterial blood rises to nearly double that of the alveolar air, and considerably above that of the external air. With intermediate percentages of CO there are intermediate rises in the arterial oxygen pressure.

It is thus evident that, although under normal resting conditions absorption of oxygen occurs only by diffusion, want of oxygen in the tissues of the body brings into play a supplementary secretory activity by which oxygen is *actively* absorbed from the alveolar air into the blood. This process is presumably analogous to that by which oxygen at a partial pressure of sometimes as much as 100 atmospheres above that in the sea water is secreted into the swim-bladder of deep-sea fishes.

It is satisfactory to find that the results by the carbon-monoxide method agree closely with those hitherto obtained by the aerotonometer method. The reasons why Fredericq and Krogh have obtained no evidence in favour of the secretion theory are also evident. Still more satisfactory is it to find that the process of absorption of oxygen by the lungs is regulated, just as is the breathing itself, in accordance with the physiological requirements of the organism. But for the secretory process the blood would be very incompletely saturated during muscular work, when five, or even ten, times as much oxygen is absorbed as during rest. During rest, on the other hand, the secretory process is not required, and would be a waste of physiological effort.

The Action of Nicotine and other Pyridine Bases upon Muscle.

By V. H. Veley, F.R.S., and A. D. Waller, F.R.S.

(Received February 22,—Read March 10, 1910.)

Nicotine, or pyridyl *n*-methyl pyrrolidine, $C_5H_4N.C_4H_7N(CH_3)$ (m.w. = 162), best known to physiologists in this country in connection with its action on sympathetic ganglia as pointed out by Langley, is generally regarded as having little or no action upon muscle.

According to recent observations of Langley the drug does, however, act upon muscle in a peculiar way, that has led him to a theoretical interpretation which we shall consider later. Our original purpose was the simpler one of comparing upon muscle the action of nicotine and allied substances.

We have made, independently, two separate series of observations, one during September, 1908, with nicotine tartrate, $C_{10}H_{14}N_2.2C_4H_6O_6.2H_2O = 498$, the other during September, 1909, with the free base, following the method described in previous communications, according to which the muscle is excited at intervals throughout observation.

In both series, with differences of detail attributable to the fact that the tartrate in solution is probably to some extent hydrolysed, the nicotine record is unmistakably characteristic, and not presented by any other substance that we have examined. Both in the case of the salt and in that of the base, the drug in moderate concentration produced :—

(1) Contracture with twitching.

(2) A first diminution of contraction not reaching to complete abolition.

(3) A subsequent increase of contraction up to, or even beyond, its original value, the muscle remaining throughout in the nicotine solution.

(4) A final gradual decline of contraction.

Typical records are as under; the "type" is characteristic and reproduces itself in *all* our records.

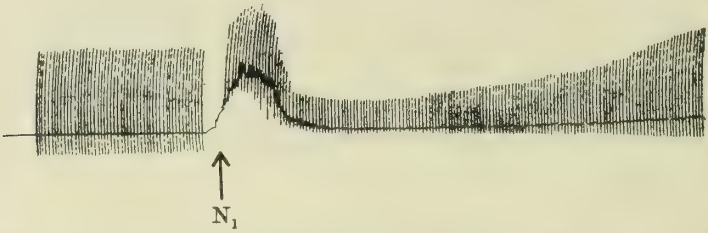


FIG. 1.—Nicotine tartrate, 0·01 per cent. (*n*/1620 as base); at N_1 a solution of nicotine tartrate, 0·01 per cent., is run in. The typical effect (twitching, contracture, and primary diminished contraction, giving way to increased contraction) is well marked.

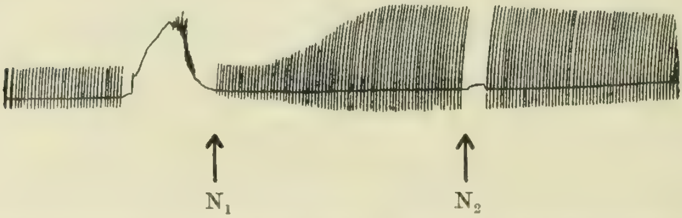


FIG. 2.—Nicotine tartrate, 0·01 per cent. at N_1. Excitation suspended from this point to the next, so as to allow the contracture and twitching to be uncomplicated by the effects of excitation; the subsequent increase of contraction is well marked. 0·1 per cent. at N_2 gives no marked effect.

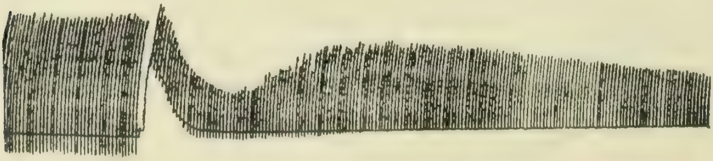


FIG. 3.—Nicotine *n*/1000 (0·016 per cent.).

Other records (not here reproduced), taken at various strengths of solution, were so much alike as to be hardly distinguishable apart, and altogether different from the records obtained with any other drugs.

Summary of Recorded Observations.

Nicotine Tartrate.

	0·1	per cent.	$n/162$	Typical effect.
	0·1	„	$n/162$	„
	0·2	„	$n/324$	„
	0·01	„	$n/1620$	„ Fig 1.
then	0·1	„	$n/162$	No effect.
	0·01	„		Typical effect.
then	0·1	„		No effect.
	0·01	„		Typical effect.
then	0·1	„		No effect.
	0·01	„		Typical effect (originally small, then large. Fig. 2).
then	0·1	„		No effect.
	0·01	„		Typical effect.
then	0·1	„		No effect.
	0·01	„		Typical effect.
	0·001	„		No effect.
then	0·01	„		Slight typical effect.

Nicotine.

0·081	„	$n/200$	Typical effect; abolition in 11 minutes; imperfect recovery after 20 minutes.
0·041	„	$n/400$	Typical effect; abolition in 15 minutes; fair recovery after 9 minutes.
0·032	„	$n/500$	Typical effect; abolition in 17 minutes; fair recovery after 5 minutes.
0·016	„	$n/1000$	Typical effect. Fig. 3.

We find that the peculiar nicotine effect may fail to present itself at lowered temperatures, and that it can be brought out into clearer evidence at higher temperature.

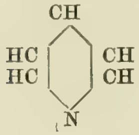
The characteristic nicotine effect cannot be produced twice in the same muscle; if, *e.g.*, it has been brought about by immersion of a muscle in a solution of 0·01 per cent. nicotine, it cannot be reproduced by a second immersion in nicotine solution of ten times the strength (fig. 2), which simply abolishes all muscular contractility.

The characteristic effect fails to appear in the case of muscles removed from frogs that had received by subcutaneous injection a lethal dose of curarine or of nicotine.

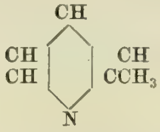
The Pyridine Bases.—The study of nicotine naturally involves that of its parent base pyridine, as well as that of methyl pyridine or picoline and of piperidine or hexahydropyridine, the active principle of pepper.

The constitutional formulæ of these several bases are as follows :—

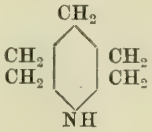
Pyridine.



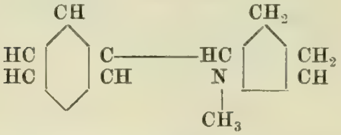
Picoline.



Piperidine.



Nicotine.
Pyridyl *n*-methyl pyrrolidine.



Of these four bases we find that, as regards their action on muscle, nicotine is the most active, and piperidine about half as active as nicotine. Picoline and pyridine have about one-tenth of the toxic power of nicotine. This, of course, is reckoning by molecules.

The characteristic nicotine effect is altogether absent from the records of the three other pyridine bases. To see whether it belongs to the pyrrolidine moiety, or is characteristic of the entire molecule of nicotine, it would be necessary to test the action of the former substance, but so far we have not been able to obtain it.

Summary of Observations.

Piperidine.

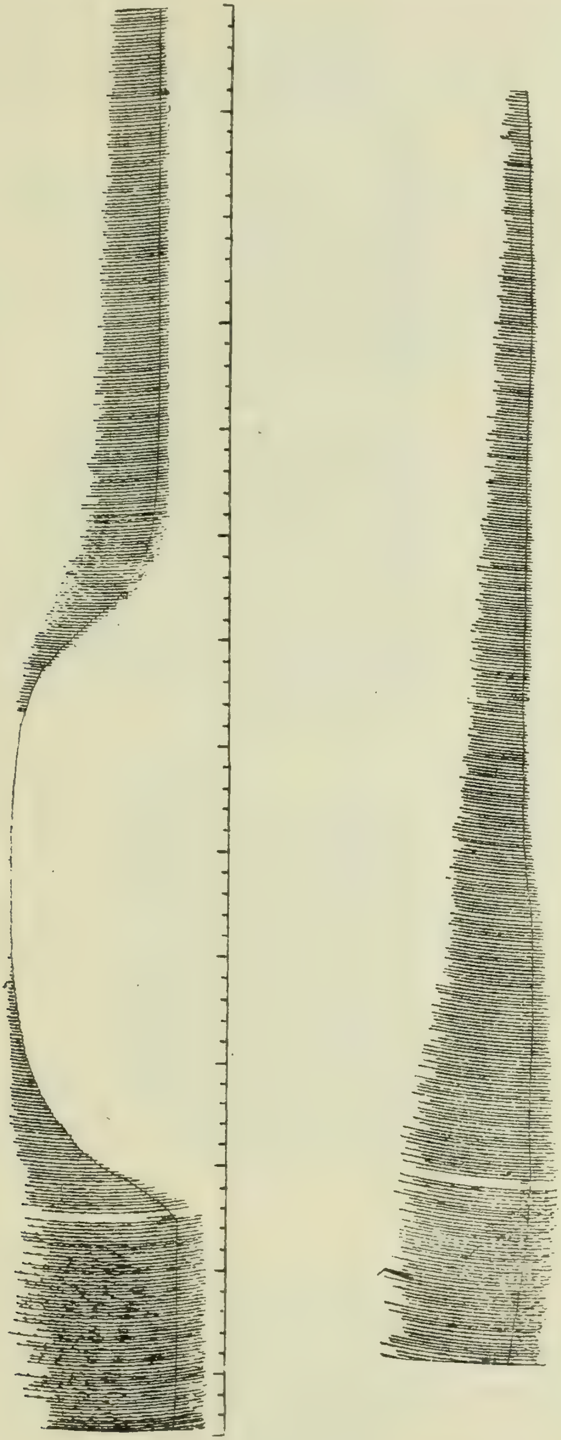
	0·085	per cent.	*n*/100......	Immediate abolition with contracture.
	0·021	„	*n*/400......	Gradual decline. (Fig. 5.)
	0·043	„	*n*/200......	Abolition in 11½ minutes with contracture. Commenced after 12 minutes' imperfect recovery. (Fig. 4.)

Pyridine. (Fig. 6.)

	0·395	„	*n*/20	Abolition in 3 minutes without contracture. Recovery begins in 5 minutes and is complete.
then	0·197	„	*n*/40	No effect.
	0·197	„	*n*/40	No effect.
then	0·395	„	*n*/20	Abolition in 5 minutes without contracture.

Picoline. (Fig. 7.)

	0·093	„	*n*/100......	No effect.
	0·186	„	*n*/50	Very gradual decline.
	0·233	„	*n*/40	Abolition in 13 minutes without contracture ; immediate recovery.
	0·465	„	*n*/20	Abolition in 5½ minutes ; immediate recovery.



Figs. 4 and 5.—Effect of piperidine upon muscular contraction. Abolition, with contracture, in $n/200$ (0·043 per cent.) solution. Gradual abolition in $n/400$ (0·021 per cent.) solution.

A comparative experiment in which *n*/20 and *n*/40 solutions of pyridine and picoline were alternately applied several times to the same muscle, showed that there was no appreciable difference between the toxic effect of these two bases.

We assign the following order of molecular toxicity to these bases, viz., nicotine = 100, piperidine = 50, pyridine = 10, picoline = 10.

The toxicity of nicotine as compared with that of some other of the poisons that we have examined during the past year can be appreciated from the following numbers, which denote relative molecular toxicity as estimated by experiments on the sartorius muscle:—

Aconitine	1000	Caffeine	12
Quinine	100	Chloroform	6
Nicotine	33	Ether	0·72
Theobromine	18	Alcohol	0·06

Thus, *e.g.*, molecule for molecule, the power of nicotine is about 1/3 that of quinine (or, weight for weight, about 2/3).

Curare and Curarine.—As regards curare and its action upon muscle we made some preliminary experiments, partly for the purpose of meeting an objection made to us that our effects as regards muscle might in part be end-plate effects, but principally in order to see whether curare in weak solution acts directly upon muscle, and to learn whether such action, if present, could be taken as an indication of the specific power of curare tested in the usual way by injection.

We compared two samples of curare. The first, a commercial curare of good repute and price (10*s.* per gramme), but relatively inactive; the second a laboratory preparation of curarine iodide, $C_{19}H_{25}N_2O.I$,* that was given to one of us several years ago by Prof. Boehm, and had been prepared by him from the last consignment of genuine "calabash curare" that was imported into Europe.† This preparation, which is the only derivative of curare exhibiting a crystalline character, has, according to Boehm, the formula $C_{19}H_{25}N_2O.I$; his analyses afforded the following average percentage: C = 53·35; H = 5·85; N = 6·17; I = 30·08.

The relative specific powers of the two preparations may be gathered from the following comparison: a pipette full (0·3 c.c.) of 1-per-cent. solution of

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* R. Boehm, "Curare in chemischer und pharmakologischer Beziehung," 'Abh. d. math.-phys. Cl. d. Königl. Sächsischen Ges. d. Wiss., Leipzig,' vol. 22, 1875, 'Das Tubo-Curare'; vol. 24, 1897, "Das Calabassen Curare," "Das Topfcurare."

† This statement is made on the authority of a statement made to me by Prof. Boehm on March 30, 1901.—A. D. W.

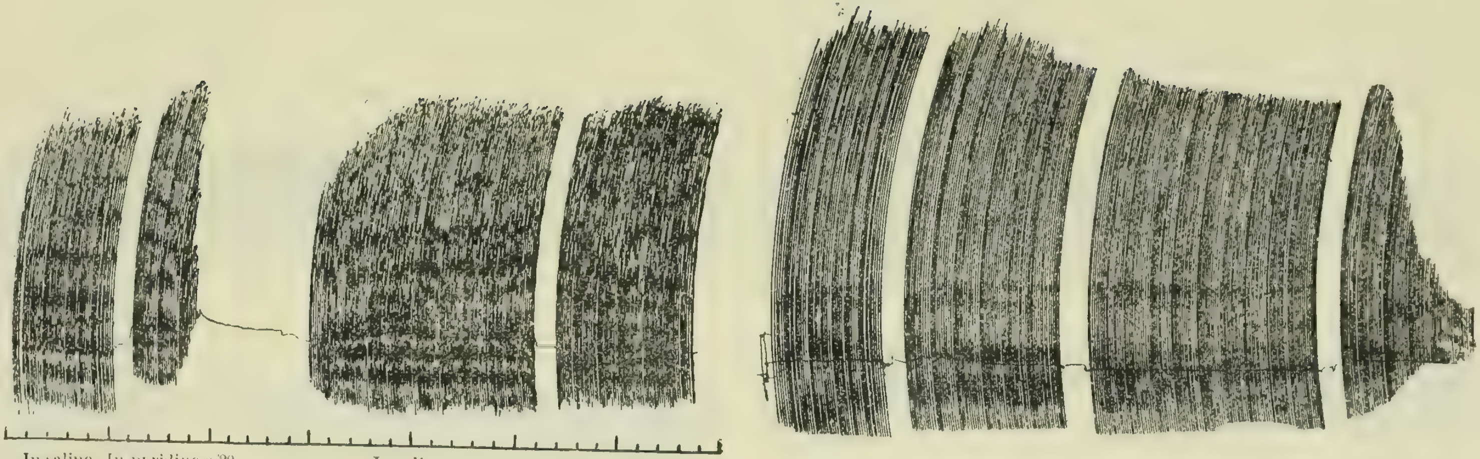

Fig. 6.—Simultaneous record of two muscles, R. and L., immersed in normal saline and in pyridine solutions, as indicated below the record. (The greater amplitude of these as compared with other records is due to the fact that we were then using levers with a greater magnification.)

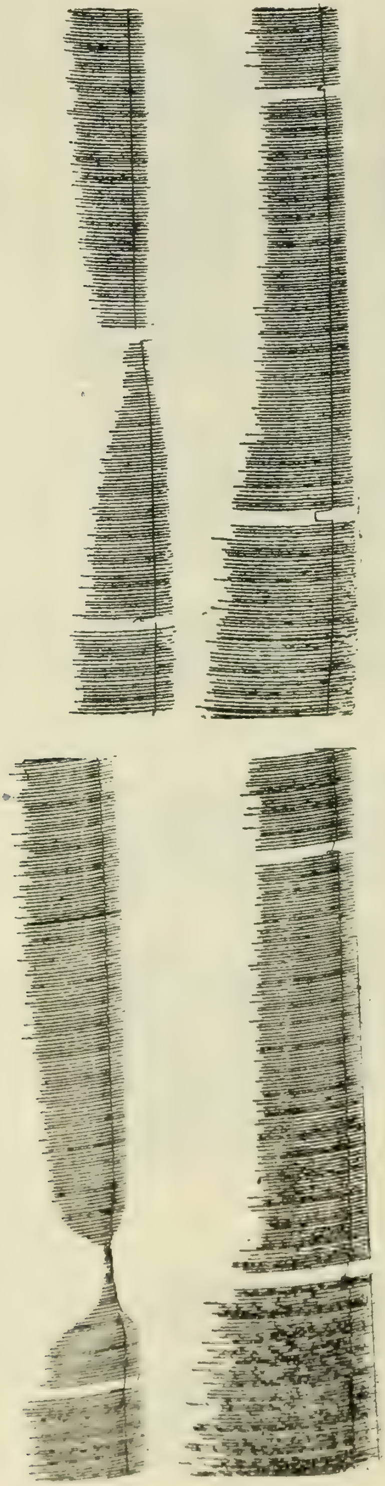

Fig. 7.—Simultaneous record of two muscles, R. and L., immersed in saline and in picoline solutions as indicated on the record.

commercial curare was required for the complete "curarisation" of an average sized frog (20 to 30 grammes); a similar effect, on a similar frog, was produced in a shorter time by the same volume of a 0·01-per-cent. solution of Boehm's preparation.* The latter was thus known to be upwards of 100 times as active as the commercial product, as judged of by its specific action as regards blocking of the impulse from nerve to muscle.

As regards direct effects upon muscle, both preparations of curare were what we are accustomed to call "slightly active," *i.e.* in 0·1-per-cent. solution the contractility of muscle was not abolished in half an hour (fig. 8). Boehm's curarine in 0·1-per-cent. solution was rather more active than the solution of commercial curare at the same concentration (but considerably less active than nicotine at 0·1 per cent. and even 0·01 per cent.); this difference of activity between the two samples of curare was much below the difference as judged by the specific "curarisation" test.

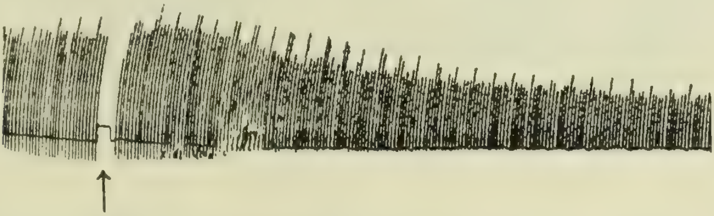

FIG. 8.—Effect of curarine iodide, 1 per 1000, or 0·0024 *n*. (Boehm.)

The Antagonism of Nicotine by Curarine.

Langley has pointed out the antagonism of nicotine by curare; our nicotine and curarine experiments are confirmatory of this antagonism, and bring out approximately a quantitative relation between them. We find by immersing muscles in mixtures of nicotine and curarine solutions in which the proportion of N : C by molecules is varied from 2 : 1 to 160 : 1, that the typical nicotine effect is unfailingly abolished when 30 molecules of nicotine are in presence of 1 molecule curarine. In this instance the nicotine was taken at $n/500$, *i.e.* well above the strength required for a characteristic effect, as, moreover, was indicated by a simultaneous control experiment. The curarine in the mixed solution was at the concentration $n/16000$, *i.e.* far below a concentration at which curarine by itself can act upon muscle. As shown above, curarine in $n/424 = 1/1000$ does not produce abolition of contraction in half an hour, yet, in the mixed solution curarine in 1/40 of that mass, altogether effaces the effect of nicotine. This is a very striking case of antagonism, and as regards the proportion between the antagonistic reagents we have understated it.

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* Langley's "curare" seems to have been weak, probably the "pot curare" that has replaced "calabash curare" in the market during the last 20 years (R.S., p. 176); he antagonises 4 milligrammes nicotine by 50 milligrammes of curare, and refers to this as being two or three times the amount required to prevent the "sciatic causing contraction of the muscle."

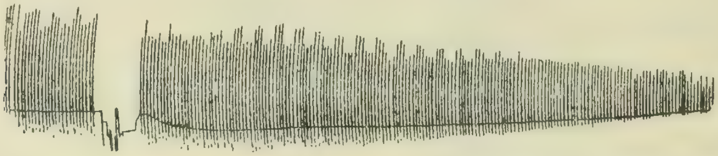

Fig. 9.—Complete abolition of the typical nicotine effect by curarine iodide, N/C = 31/1.

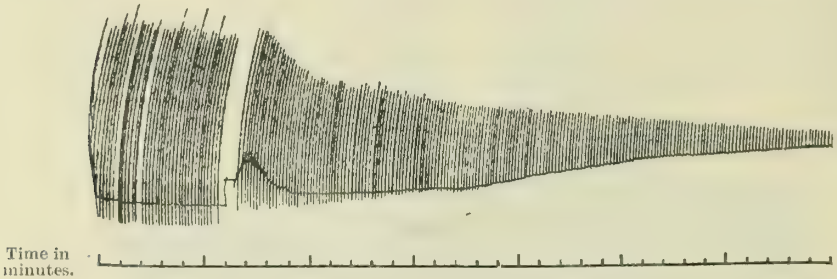

Fig. 10.—Almost complete abolition of the typical nicotine effect by curarine iodide, N/C = 160/1.

Taking the mixed solution with curarine at five times the dilution, *i.e.* with curarine at *n*/85000 and nicotine as before at *n*/500, there is still a very considerable if not absolutely complete abolition of the characteristic nicotine effect. In this case the proportion by molecules is 160 : 1.

That this antagonism is of a peculiar, and, so to speak, specific character, is borne out by experiments with other drugs, where we find that far greater proportional mass is required for the abolition of the characteristic nicotine effect.

We have tested in this connection the influence of cinchonamine. Taking, *e.g.*, a mixture of nicotine at *n*/1000 *plus* cinchonamine *n*/2000, we obtained a record exhibiting the characteristic effects of nicotine and cinchonamine, but in which the nicotine effect was less in the presence of cinchonamine than it would have been in its absence, and in which the cinchonamine effect was lessened by the nicotine. This appears to us to indicate a state of subdivision, the muscle stuff being conjointly occupied by the two bases, nicotine and cinchonamine, as in the ordinary case of subdivision of an acid between two bases; but the case of nicotine and curarine cannot be brought under the same category.

We have also tested mixtures of nicotine and strychnine, with proportions of nicotine to strychnine = 1 : 2·5 (nicotine *n*/1000 + strychnine *n*/400); the nicotine effect was entirely replaced by the strychnine effect. In the proportion N : S = 4 : 1 (nicotine *n*/1000 + strychnine *n*/4000) the nicotine effect is not completely abolished.

We have also tested mixtures of nicotine and aconitine. Taking a mixture of nicotine *n*/1000 and aconitine *n*/40000 (N : A = 40 : 1), we found that the nicotine effect was absolutely unaltered. With a mixture of nicotine *n*/1000 and aconitine *n*/2000 the nicotine effect was abolished, the record being what we should term a modified aconitine record. With nicotine *n*/1000 and aconitine *n*/20000 the nicotine effect was just visible, while with nicotine *n*/1000 and aconitine *n*/10000 the nicotine effect was abolished.

We also tested mixtures of nicotine and quinine. With a mixture of nicotine *n*/1000 and quinine *n*/1000, we obtained a record indicative of a subdivision, with a considerable predominance of the quinine over the nicotine effect. In the proportion nicotine *n*/1000 : quinine *n*/5000 (= 5 : 1) the nicotine effect was not abolished, but in the proportion nicotine *n*/1000 : quinine *n*/10000 (= 10 : 1), the nicotine effect is unaltered; that is to say, the effect of 10 molecules of nicotine is not overcome by a molecule of quinine, nor that of five molecules completely so, whereas one molecule of nicotine is overpowered by one molecule of quinine.

Thus in none of these cases could we regard nicotine as antagonised as by curarine. The results were such as to indicate either a subdivision of the muscle protein between two bases or the displacement of a weaker base by a stronger. Whereas in the case of curarine we have a substance with little toxic action on muscle itself, yet of which one molecule was sufficient to very nearly abolish the effect of 160 molecules of nicotine, but it required 1 molecule of strychnine or quinine to overpower 1 molecule of nicotine; and 1 molecule of the most powerful poison we have used, viz., aconitine, could overpower at most 10 molecules of nicotine.

Langley considers that both nicotine and curare combine with some substance in the muscle itself, and that it is unnecessary to resort to the assumption of an additional effect on nerve-endings; he finds, indeed, that the antagonism between these two poisons occurs in muscle of which the nerves have completely degenerated.

We find it difficult, in presence of the fact that curarine (which acts powerfully on nerve-endings, feebly upon muscle) does very readily antagonise the characteristic nicotine effect (the seat of which is now in question), to avoid the conclusion that such nicotine effect takes place at the nerve-ending, and is there antagonised by curarine.

Note 1.—A. V. Hill,[*] working in Langley's laboratory and according to his method, used extremely dilute nicotine solution, viz., 0·00003 per cent., *i.e.* approximately $n/500000$ (we generally used solutions of 1000 to 500 times this strength, viz. $n/1000$ and $n/500$).

For the antagonism of nicotine by curare, Hill, working with the *rectus abdominis* muscle, gives 0·00006 per cent. (*i.e.* $n/25000$) nicotine as antagonised by a 0·05-per-cent. solution of curare, and 0·00001 per cent. (*i.e.* $n/150000$) as antagonised by a 0·005-per-cent. solution of curare.

We used in our antagonism an $n/500$ (0·03 per cent.) of nicotine with curarine amounting to $n/10000$ (0·0026 per cent.) and $n/85000$ (0·0005 per cent.).

Following the same method, he determines by Arrhenius' formula the temperature constant for nicotine = 17,340, which corresponds with the constant $m = 31·4$ by Esson's formula.

Note 2.—Several years ago, one of us (A. D. W.) made a considerable number of experiments on the isolated frog's heart, for the purpose of comparing the activity of nicotine with that of pyridine. The comparison failed by reason of the irregular effects exhibited by the heart. The failure is worth mention in the present connection in illustration of our opinion that isolated muscle affords a good physiological reagent for the comparison of toxicity. Thus, *e.g.*, the relative power of ether and chloroform can be estimated with far greater accuracy and uniformity of results on isolated muscle than on the isolated heart.

In a recent paper we have given the temperature constants by Esson's formula for

Alcohol............ $m = 20·8$,
Chloroform ...... $m = 14·3$,
Quinine............ $m = 26·7$;

these correspond with the Arrhenius constants:—11,550, 7930, and 14,820 respectively.

But Hill's paper does not contain any data or records by which it would be possible to compare his results with our own. His conclusion is, however, the same as ours, viz. that the high value of the constant is evidence of a chemical combination between drug and muscle.

Arrhenius' formula, as expressing variation of rate of chemical change with temperature, has been generally adopted by writers on Physical and on Physiological Chemistry.

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[*] 'Journal of Physiology,' vol. 39, p. 361.

It is, however, rather difficult to follow the line of reasoning on which it is based; further, the numbers in the units and the tens of the derived constant μ have no real significance.

On a comparison of the constant m of Esson's equation:—

$$\log K_{T_1} - \log K_{T_0} = m(\log T_1 - \log T_0) \qquad (1)$$

with that of μ in the Arrhenius formula, which may be written with the same terms on the left-hand side, thus:—

$$\log K_{T_1} - \log K_{T_0} = \left(\frac{\mu}{2}\,\frac{T_1 - T_0}{T_1 T_0}\right)\log e \qquad (2)$$

(K_{T_1}, K_{T_0} = factors of chemical change at absolute temperatures T_1, T_0 respectively), it appears that $m = \mu/555$, or $\mu = 555\,m$.

The values of the constants m and μ obtained by the two formulæ for the action of the drugs: alcohol, chloroform, quinine (Veley and Waller), and nicotine (A. V. Hill) are here set forth:—

Substance.	Temp. range.	Values of m.	Values of μ.	Ratio.
Alcohol	7° to 24°	20·8	11,570	1 : 556
Chloroform	7° „ 24°	14·3	7,700	1 : 535
Quinine	7° „ 25°	26·7	14,950	1 : 559
Nicotine	17° „ 27°	31·4	17,340	1 : 553

The respective rates at the highest and lowest temperatures respectively have been taken for the purpose of the above comparison; a slight experimental error in one of them would account for the rather low value found in the case of chloroform.

The range of temperature (about 20°) within which the derived constants of the two formulæ are compared may at first sight appear to be rather limited; but this equally applies in practice to most chemical changes which cannot be measured as a rule with any degree of accuracy beyond a limiting range of 30°.

Preliminary Note on the Origin of the Hydrochloric Acid in the Gastric Tubules.

By Mabel Purefoy FitzGerald (Oxford).

(Communicated by Prof. A. B. Macallum, F.R.S. Received March 7,—Read March 10, 1910.)

(From the Biochemical Laboratory of the University of Toronto.)*

The place of origin in the peptic glands of the hydrochloric acid of the gastric juice has been, during the last 60 years, a question the solution of which has been directly attempted by a number of observers whose efforts have on the whole been unsuccessful.

The first to attack the question was Claude Bernard, who sought to localise the acid formation in the glands by injecting into the blood-vessels of a rabbit, first a solution of lactate of iron and thereafter one of potassium ferrocyanide. When the two solutions mingle in the circulating fluid and tissues of the body the mixture should, he assumed, only give the Prussian blue reaction in the presence of a free acid, and, in consequence, when diffused through the gastric glands, it should develop the blue reaction where the hydrochloric acid is formed. The assumption is not quite correct. The mixture of the two solutions, as soon as it is made, in the test-tube, develops Prussian blue even when the purest reagents are employed and, therefore, though the addition of an acid greatly enhances the blue reaction, the latter is not infallible evidence of the occurrence of a free acid. With this method, however, Bernard found a blue deposit on the surface of the mucosa in the lesser curvature of the stomach, but no trace of blue in the interior of the mucosa. This result left the question undecided.

Other observers also employed this method, but with a like result. Even with aniline dyes which, reacting with acids, give a different colour, the histological elements concerned in the formation of the acid could not be determined.

All the facts known regarding the structure of the peptic tubules in Mammals seem to indicate that the cells engaged in the formation of the acid are those known as parietal.

This has now been directly confirmed, and by the use of Claude Bernard's method. In this, however, the citrate of iron and ammonia replaced the lactate salt which he used. Mixtures of solutions of the double citrate salt

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* Research carried out while holding a Travelling Fellowship of the Rockefeller Institute for Medical Research, New York, 1908.

and potassium ferrocyanide give, even after standing for 24 hours, no evidence of the formation of Prussian blue, nor is the latter produced in the mixtures when either sodium dihydric phosphate or carbon dioxide dissolved in excess is present. On the other hand, the addition of very dilute acids, organic or inorganic, suffice to develop at once a marked Prussian blue reaction. In the case of hydrochloric acid, a concentration of 36 parts of acid in 100,000 (or 0·036 per cent.) gives, with an equal volume of the mixture, a distinct blue, and as the acid in the gastric juice is much more concentrated than this, a mixture of equimolecular solutions of the double salt and potassium ferrocyanide ought, when injected hypodermically or intravenously, to indicate minutely the structures in the gastric mucosa which are concerned in the production of hydrochloric acid. Hypodermic injections of the mixture in rabbits and guinea-pigs give results which fully bear out this assumption.

Within a few hours after the injections, a more or less circumscribed greenish deposit was found adhering to the mucosa of the lesser curvature adjacent to the œsophageal opening. An examination of sections of the underlying tissue revealed, in some instances, no evidence of any blue compound in the gland tubules or in the crypts into which they opened, but in one rabbit the Prussian blue was found at isolated points in the gland tubules and in the crypts into which they opened. In this case only rarely did the reaction appear throughout the length of the upper two thirds of the lumen of a tubule. Much more often it was confined to the third of the lumen next the crypt, while it never obtained in the lowest third.

Here and there from these deposits in the lumina there branched out lines in blue, which penetrated the parietal cells. As a rule there was only one branch to each cell, but occasionally as many as three or more were found to penetrate a cell. Each branch, immediately on entering the cell, usually ramified, and the branchlets, penetrating the depths of the cell, in some cases curved around the nucleus to reach the cytoplasm on its far side. The blue twigs did not appear to touch the nucleus. Local aggregations of the blue deposit in these canaliculi gave the latter at times a beaded appearance.

The cytoplasm itself, apart from the ramifications of the blue canaliculi, was as a rule free from colour. The exceptions implied in this were cells in which, under the most favourable daylight illumination, an exceedingly faint, almost imperceptible, blue appeared throughout the cytoplasm. In some cases it was uncertain whether this was due to a scattering of the light reflected from the blue material in the canaliculi, but in the other cases the faint blue appeared to indicate that there is free hydrochloric acid in the cytoplasm.

It would seem that however the acid is held in the cytoplasm, whether in the free (ionised) or in the "masked" (un-ionised) condition, it is not always secreted into the canaliculi. In some cases, notably in certain rabbits, it was liberated only on the surface of the cell remote from the lumen of the tubule. In this case there was no blue deposit in the lumina of the tubules or in the canaliculi of the parietal cells, but in the lymph capillaries immediately adjacent to the tubules there was a blue granular deposit which revealed the course of the capillaries. The lymph cells in the course of the larger channels also contained in their cytoplasm a blue granular deposit. Sometimes, further, the contents of the blood capillaries in such preparations here and there gave a blue reaction. The explanation which may be advanced for these results is that the cytoplasm of the parietal cells, perhaps under the toxic effect of the potassium ferrocyanide, has lost some of its osmotic properties and the direction of elimination of the acid is changed; in other words, the parietal cells in this case have, as it were, lost the sense of direction.

The observations briefly summarised here will, with others of a cognate character, eventually appear in a more complete communication.

Studies on Enzyme Action. XIII.—*Enzymes of the Emulsin Type.*

By Henry E. Armstrong, F.R.S., and Edward Horton, B.Sc.

(Received March 3,—Read March 10, 1910.)

[*International Catalogue of Scientific Literature.*

Authors' title slip :—D. Q.
Subject slips :—

D 1850 Phaseolunatin—a β-glucoside. Hydrolysis by enzymes.
D 6300 Hydrogen cyanide. Estimation of
D 8014, Q 1240 Enzymes of the emulsin type. Action on Fischer's glucoside, amygdalin and phaseolunatin.
D 8014 Cellase. Behaviour towards cyanophoric glucosides of.]

In the previous communication of this series (No. XII, 1908, B, Vol. 80, pp. 321—329*) the contention was advanced that "emulsin" prepared from sweet almonds contains at least three distinct enzymes, viz., *amygdalase*, a *β-glucase* and *gluco-lactase*: the first of these, amygdalase, being the enzyme by which amygdalin is resolved into glucose and Fischer's glucoside, β-glucase that by which β-glucosides are hydrolysed, gluco-lactase that which hydrolyses milk sugar.

Meanwhile the striking discovery has been made by Rosenthaler† that emulsin has the property of inducing the formation of dextro-rotatory benzylidenecyanhydrol—such as may be obtained from amygdalin—from benzaldehyde and hydrogen cyanide; this raises the question whether there may not also be present in "emulsin" a specific enzyme capable of operating on the cyanophoric radicle of amygdalin and of resolving it into hydrogen cyanide and benzaldehyde. To avoid periphrasis this hypothetical enzyme may be spoken of as *benzcyanase.*

In continuing the study of the problem presented by almond "emulsin," we have been led to contrast it with active preparations producing somewhat similar effects derived from other sources, our hope being that we should sooner or later find one or other specific enzyme occurring alone. One of the preparations which has attracted our special attention is that derived from *Phaseolus lunatus,* the seed of which, as Dunstan and his co-workers have shown, contains a cyanophoric glucoside that is resolved under the influence of the enzyme present in the beans into glucose, acetone and hydrogen cyanide and is therefore allied to Fischer's glucoside in the manner shown in the following formulæ :—

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* *Correction, paragraph* 4, *p.* 325 : *for* β-methylglucoside *read* α-methylglucoside.

† 'Biochemische Zeitschrift,' 1908, vol. 14, pp. 238—253; 1909, vol. 17, pp. 257—269.

$$Me_2C(CN).O.C_6H_{11}O_5 \qquad PhHC(CN).O.C_6H_{11}O_5$$

The statements made with regard to this glucoside and the associated enzyme by Dunstan, Henry and Auld in their several communications to this Society* are of a very striking character.

In their valuable summary account of the cyanophoric glucosides, read at the meeting of the British Association in York, in 1906,† Dunstan and Henry point out that the enzymes found in *P. lunatus* seeds are of special interest. To quote almost their words :—

The mixture prepared from the beans contains at least two enzymes, one of the emulsin type and the other of the maltase type ; it is the latter which attacks phaseolunatin. Examination of the dextrose produced by the hydrolysis of phaseolunatin by the maltase-like enzyme present in the beans shows that it is the α-isomeride, so that phaseolunatin is an α-glucoside and may therefore be represented as the α-dextrose ether of acetonecyanohydrin.

In order that these statements may be understood, it is desirable to give a short account of the work on which they are based. It appears that the glucoside termed *phaseolunatin* by Dunstan and Henry, by whom it was described in 1903, was first isolated, in 1891, from young flax plants, by Jorissen and Hairs, by whom it was termed *Linamarin*—a name for which, therefore, priority may be claimed. The statement made by Dunstan and Henry, in 1903, was that the enzyme of *Phaseolus lunatus* readily hydrolyses amygdalin, salicin and phaseolunatin. As the latter was also hydrolysed by the emulsin of sweet almonds, it was probable that the enzyme of *Phaseolus lunatus* is emulsin.

Jorissen and Hairs, who examined the enzyme contained in flax seedlings, found—to quote Dunstan—that whilst it had the property of hydrolysing both linamarin and amygdalin, the emulsin of almonds was incapable of decomposing linamarin.

Jouck, however, states, in opposition to Jorissen and Hairs, that linamarin *is* decomposed by the emulsin of almonds.

The enzyme extracted from flax seeds was found by Dunstan, Henry and Auld to have a range of activities similar to that of the emulsin of almonds : it readily hydrolysed amygdalin and salicin.

They were led to conclude that probably the same enzyme is contained in *Phaseolus lunatus* seed and in flax seed and that this enzyme is of the emulsin type—*i.e.* it appears to hydrolyse β-glucosides—and exhibits similar activities ; but they recognised that it presented certain well-marked differences from emulsin.

These differences were clearly defined in a later communication, in which they withdrew the statement that phaseolunatin is decomposed by emulsin of almonds, explaining that this had been made on the basis of "a single experiment in which prussic acid was undoubtedly liberated when a commercial emulsin preparation was added to an aqueous solution of the glucoside. This experiment has, however, been repeated frequently, using several different commercial emulsin preparations as well as emulsin prepared by ourselves from sweet almonds and in no case have we been able to observe again the formation of prussic acid within a reasonable time, though in every case prussic acid was liberated on the further addition of the mixture of enzymes prepared from the beans of *Phaseolus lunatus* or from flax or cassava."

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* Dunstan and Henry, 'Roy. Soc. Proc.,' 1903, vol. 72, pp. 285—294 ; Dunstan, Henry and Auld, *ibid.*, B, 1906, vol. 78, pp. 145—152 ; Dunstan, Henry and Auld, *ibid.*, B, 1907, vol. 79, pp. 315—322.

† Dunstan and Henry, B. A. Report, 1906, p. 150.

Finding that almond emulsin decomposed amygdalin and salicin but not phaseolunatin, whilst the "enzyme" associated with this last glucoside affected all three, they were led to think that perhaps two enzymes were present in Phaseolus beans, etc.; the experiments they then made led them to the conclusion that this was the case and that the one enzyme was of the emulsin type, the other of the maltase type. In fact, as before stated, they were led to think that phaseolunatin is an α-glucoside.

The evidence on which this conclusion was based was twofold: in the first place, in comparative experiments with methyl-α-glucoside and phaseolunatin, they found that both compounds were hydrolysed in contact with yeast extract and still more readily when the solution was digested with dried yeast. This led them to apply the method developed by E. F. Armstrong, involving the determination of the forms in which dextrose is liberated on hydrolysing the glucosides by noticing the rotatory power of the solution and then adding sufficient alkali to determine the rapid formation of an equilibrated mixture of the two isomeric forms of glucose.

Finding that alkali caused an increase in the lævorotatory power, they came to the conclusion that glucose was liberated in the α-form.

The conclusion finally arrived at was that the extract prepared from Phaseolus beans contains an α-enzyme which if not identical with yeast maltase is of the same type; and that it also contains a β-enzyme identical with or similar to emulsin. These conclusions were arrived at, it should be mentioned, in face of the known fact established by Henry and Auld that yeast contains an emulsin-like enzyme.*

The results arrived at by Dunstan and his co-workers are so remarkable, it is so difficult to reconcile their views in all respects with existing opinion, they cross our field of investigation in so many ways, that we have felt compelled to include the Phaseolus glucoside and its attendant enzyme in our programme of work. The results we have arrived at are such as to lead us to conclusions very different from those we have referred to.

In our opinion, the glucoside extracted from *Phaseolus lunatus* seeds is not an α-glucoside and we are convinced also that the hydrolyst associated with it is not of the α-type; the evidence appears to us to be such as to justify the conclusion that both the glucoside and the correlated enzyme belong to the β-series.

Stress may be laid on the fact that in all the experiments described (except some of those with methyl α-glucoside) the concentration of the glucoside in the solutions studied was initially one-fifth molecular. We are strongly of the opinion that concordance will not be apparent between the results of different workers, even when using the same hydrolytes and the same enzymes, until agreement be arrived at to carry out experiments under "molecularly" comparable conditions. The concordance which probably exists between apparently conflicting results published by different authors is, we think, often hidden by the differences in the conditions under which observations are made.

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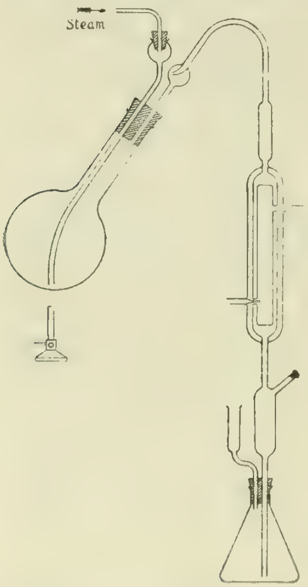
* 'Roy. Soc. Proc.,' 1905, B, vol. 76, p. 568.

The Estimation of Hydrogen Cyanide.—In most cases the determination of the activity of the enzyme preparations considered in this communication has involved the estimation of hydrogen cyanide; the method we have adopted is a simplification of that referred to in No. XII of these studies (p. 329). Instead of filtering off the precipitate of silver cyanide in the manner there described, after running the measured sample (10 c.c.) into a flask (300 c.c.) containing the solution of silver nitrate (15 c.c.—N/5) and then adding sodium acetate (15 c.c.—N/5), if benzaldehyde be present we pass steam into the mixture during 2—3 minutes until the aldehyde is volatilised and then add to the cooled liquid, ammonia (5—10 c.c.) and afterwards a solution of sodium chloride (4 c.c. – N/1).

The flask is then connected to a condenser and excess of a saturated solution of tartaric acid (about 20—25 c.c.) is poured in through a thistle funnel (see figure). The liquid is heated to the boiling point and a current of steam passed in during 15 minutes, the distillate being collected in a slight excess of an N/5 solution of caustic potash. The distillate is titrated with an N/20 solution of silver nitrate. An estimation can be effected in 30 minutes. The excessive frothing which often occurs when the liquid is distilled may be prevented by adding a few drops of olive oil.



To compare the method with the modification of Fordos and Gelis' method adopted by Dunstan, Henry and Auld, amygdalin was hydrolysed by emulsin and the amount of hydrogen cyanide liberated was then estimated by both methods.

A solution of 20·451 grammes of amygdalin in 200 c.c. of water containing 10 c.c. of the solution of emulsin was kept at 25°. At intervals of (usually) an hour 22 c.c. were withdrawn and introduced into a flask containing a drop of concentrated sulphuric acid, to stop the action of the enzyme. From this sample two portions, each 10 c.c., were taken: in one the cyanide was estimated as described above; the other was added to excess of a solution of sodium hydrogen carbonate and either titrated directly with an N/50 solution of iodine, or it was diluted and an aliquot portion titrated. The following results were obtained:—

Time.	Volume (c.c.) of		Weight (gramme) of prussic acid.		Percentage of hydrolysis.	
	Silver solution.	Iodine solution.	From silver.	From iodine.	From silver.	From iodine.
Hours.						
1	4·15	40·9	0·01127	0·01143	20·9	21·1
2	6·95	66·0	0·01888	0·01844	34·9	34·1
3½	9·6	91·5	0·02608	0·02557	48·3	47·3
4	10·7	94·5*	0·02907	0·02641	53·8	48·9*
5	11·95	105·0*	0·03247	0·02935	60·1	54·3*
6	—	111·5*	—	0·03116	—	57·7*
24	18·3	174·0	0·04972	0·04863	92·0	90·0

* In these cases the titrations were carried out on the following day; the results are given as showing that it is necessary to titrate at once with iodine to obtain correct values.

A second experiment, carried out with the same quantities of glucoside and enzyme under similar conditions, gave the following results:—

Time of action.	Volume (c.c.) of			Weight (gramme) of prussic acid.		Percentage of hydrolysis.	
	Silver solution.	Iodine solution.		From silver.	From iodine.	From silver.	From iodine.
Hours.							
1	3·7	37·3		0·01005	0·01021	18·6	18·9
		(*a*)	(*b*)		(*a*)		(*a*)
2	6·25	61·5	60·0	0·01698	0·01683	31·4	31·1
3	8·25	81·0	80·5	0·02241	0·02217	41·5	41·0
4	9·8	95·5	94·5	0·02663	0·02614	49·3	48·4
5	11·1	107·5	105·5	0·03016	0·02942	55·8	54·4
6	12·2	118·5	116·0	0·03315	0·03243	61·3	60·0
7	13·0	129·25	127·5	0·03532	0·03537	65·5	65·5
24	17·45	174·4		0·04741	0·04773	87·7	88·3

The titrations giving the values marked (*a*) in the third column were effected in the absence of starch, those marked (*b*) in its presence. It appears that this indicator gives erroneous results when used under conditions such as were observed by us. We noticed, in fact, that the coloration produced was very evanescent and that, unless the first appearance of a colour which did not vanish immediately on shaking were taken as indicating the end of the change, no definite result could be obtained. In all the experiments, except the first in the first table and the first and last in the second, an aliquot part of the sample was titrated with iodine. As the experiments in which the whole of the solution was titrated are also those in which the results afforded by the iodine method are higher than the results on using silver nitrate, it would appear that there is some connexion between the two facts. We do not, however, understand why this should be the case.

We are led by our experiments to conclude that in estimating small amounts of hydrogen cyanide such as were dealt with by Dunstan, Henry and Auld their method is preferable; but that in estimating the much larger amounts dealt with by us, the silver method may be used with equal advantage. The iodine method has the disadvantage that the titrations to be of any use must be effected immediately the sample is taken; this necessity restricts the use of the method to the daytime, unless starch be used as an indicator: as we have stated, this has given erroneous results in our hands.

Action of the "Enzyme" from Almonds on the Phaseolus Glucoside.—Our experiments show that the glucoside is *invariably hydrolysed* by almond-emulsin, although to a very minor extent; the conflicting results of different workers must be attributed to the use of enzymes of varying degrees of activity. In our experience, commercial emulsin is a material of variable activity; it is usually weaker than the extract prepared in the manner described in our previous communication.

Equivalent M/5 solutions of the Phaseolus glucoside and of amygdalin, containing per 100 c.c. 20 c.c. of a solution of almond-emulsin (No. XII, p. 324) were hydrolysed, the one to the extent of 2·25 per cent., the other to the extent of 94 per cent., in 24 hours at 25°. At 37° the solution of the Phaseolus glucoside was hydrolysed to the extent of only 4·5 per cent. in 24 hours and of 16·5 per cent. at the end of seven days; apparently, the almond extract contained a very minute proportion of enzyme capable of hydrolysing this glucoside.

Action of the Phaseolus Enzyme on Amygdalin.—A fact not brought out by any previous observer is that the enzyme extracted from Phaseolus seeds* acts but slightly on amygdalin.

A solution (M/5) of amygdalin, containing 20 c.c. of a solution of the enzyme prepared from Phaseolus seeds was hydrolysed only to the extent of 2·5 per cent. in 24 hours at 25°, whilst 4 per cent. underwent change in 24 hours at 37° and 12 per cent. in seven days.† A similar solution of an equivalent amount of the Phaseolus glucoside was hydrolysed by the correlated enzyme to the extent of 50·5 per cent. in 24 hours at 25° and of 77·5 per cent. in the same time at 37°.

If, therefore, the view that the Phaseolus enzyme contains both an α- and a β-glucase be accepted, this remarkable reciprocal behaviour of the two enzymes to the two hydrolytes must be taken as an indication that almond-emulsin also contains both an α- and a β-enzyme; moreover, that the relative proportions in which the two forms are present are reversed in the two enzymes. On this supposition however, as almond-emulsin is without action on methyl-α-glucoside, the α-enzyme it contains must be different from that present in yeast maltase!

Action of the Phaseolus Enzyme on Methyl-α-glucoside and on Maltose.—From the statement made by Dunstan, Henry and Auld that yeast maltase decomposes methyl-α-glucoside and maltose more rapidly than does the enzyme of *Phaseolus lunatus*, it is to be inferred that these two compounds are hydrolysed by the Phaseolus enzyme. We have not been able to detect any trace of action, even when the substances were allowed to remain in contact during several weeks.

In a preliminary experiment in which an M/5 solution of methyl-α-glucoside containing 20 c.c. of phaseolunatase solution per 100 c.c., was heated at 25°

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* Our preparation was made from ground Java beans, some of which were black, others various shades of brown and a few white. The precipitate produced by alcohol in the aqueous extract, which had been exposed over potash to remove hydrogen cyanide, was shaken up with about six times its weight of water.

† Experiments have been recorded by Auld ('Chem. Soc. Trans.,' 1908, vol. 93, p. 1266) which appear to show that the Phaseolus enzyme acts somewhat readily on amygdalin—to the extent of as much as 34·5 per cent. within an hour.

[The discordance between this résult and our own is considerably reduced if allowance be made for the different proportions of the materials used; nevertheless, the value is in advance of any we have obtained.—*April* 9.]

during 24 hours, the initial optical rotation was 9°·75 and the final value 9°·78. An experiment was then made in the following manner:—25 c.c. of a 2M/5 solution of methyl-α-glucoside having been measured into each of three 50-c.c. Jena flasks and 20 c.c. of water into one, 10 c.c. into the second but none into the third, 5 c.c. of the phaseolunatase solution was added to the first, 15 c.c. to the second and 25 c.c. to the third. Three other solutions were prepared in the same way but from a solution of the glucoside only half as strong as that first used. From each of these six mixtures a sample of 10 c.c. was withdrawn immediately after adding the enzyme and transferred to a 50 c.c. measuring-flask containing a drop of a solution of sodium hydroxide to stop the action of the enzyme. All the operations were carried out with the liquids at 25°. The main bulk of the solutions was kept at 25° during 14 days and then at 37° during a further 14 days. At intervals of seven days, of 14 days and of 28 days, a sample measuring 10 c.c. was removed from each solution and the action stopped by soda. Each sample was diluted to 50 c.c. and its reducing power was then determined by the method described by Brown, Morris and Millar (' Chem. Soc. Trans.,' 1897, vol. 71, p. 278). The following table gives the weights of copper reduced by 20 c.c. of each of the diluted samples:—

Concentration of the glucoside.	Volume of enzyme solution per 100 c.c. of acting liquid.	Weight (gramme) of copper.			
		Initial sample.	Sample after 7 days at 25°.	Sample after 14 days at 25°.	Sample after 14 days at 25° and 14 at 37°.
M/5	10	0·0037	0·0026	0·0022	0·0012
M/5	30	0·0006	0·0016	0·0006	0·0001
M/5	50	0·0007	0·0004	0·0001	0·0004
M/10	10	0·0026	0·0016	0·0009	0·0001
M/10	30	0·0010	0·0006	0·0008	0·0004
M/10	50	0·0002	0·0026	0·0001	0·0001

As a correction involving the deduction of 0·0035 gramme of copper has to be made in each case, on account of the reduction which takes place when the Fehling's solution alone is carried through the series of operations involved in making the determinations, it is obvious that not the slightest amount of methyl-α-glucoside was hydrolysed.

It might be urged that the exposure of the enzyme at a temperature of 37° during so long a period as 14 days would be likely to diminish its activity considerably, if not to destroy it; consequently that the prolongation of the experiments over so long a time proves little or nothing. To decide this point, 5 c.c. was withdrawn from each of the six flasks, each sample being put into a separate 30-c.c. flask; to each flask was then added 5 c.c. of an M/2 solution of phaseolunatin and 2·5 c.c. of water, thus giving six M/5 solutions of phaseolunatin containing respectively 4 c.c., 12 c.c., 20 c.c., 4 c.c., 12 c.c., and 20 c.c. of the original solution of phaseolunatase per 100 c.c. These solutions were maintained at 25° during 24 hours and the amount of hydrolysis effected was then determined by estimating the amount of hydrogen cyanide liberated. The percentages of phaseolunatin decomposed were as follows:—

Volume (c.c.) of original enzyme solution present per 100 c.c.	Percentage of glucoside hydrolysed.
4	9·0
12	28·25
20	46·5
4	6·0
12	29·0
20	40·7

As an M/5 solution of phaseolunatin containing 20 c.c. of the same phaseolunatase solution per 100 c.c. was hydrolysed to the extent of 45·25 per cent. (*i.e.* 2·26 per cent. per 1 c.c. per 100 c.c.) in 24 hours at 25°, it is evident that the enzyme was practically unaffected by contact with M/5 or M/10 methyl-α-glucoside solution during 14 days either at 25° or at 37°.

Action of Phaseolunatase on Maltose.—In a preliminary experiment the reducing power of an M/5 solution of maltose, containing 10 c.c. of the solution of the phaseolunatase per 100 c.c., was determined initially and after 24 hours at 25°

2 c.c. of the liquid reduced initially 0·1568 gramme of copper.
2 c.c. „ finally 0·1509 „ „

Since any hydrolysis of the maltose would increase the reducing power of the solution, it must be concluded that none had taken place.

An attempt was made to carry out a series of experiments similar to that with methyl-α-glucoside. It was impossible, however, to filter off the reduced cuprous oxide in any reasonable time, owing to the clogging of the filter by the precipitated proteid; so the experiments had to be abandoned.

It appears to us that the mistake Dunstan, Henry and Auld made was to assume that they were dealing with yeast maltase and to overlook the possible presence in their yeast of an enzyme capable of hydrolysing β-glucosides.

Action of Yeast Extract on the Phaseolus Glucoside.—The proof that the Phaseolus enzyme is without action on methyl-α-glucoside and on maltose is sufficient to show that it is neither α-glucase nor maltase; it is nevertheless conceivable that yeast has an action on the glucoside in virtue of the presence of *amygdalase*, the enzyme characterised by Caldwell and Courtauld, although this is in no way probable in view of the fact that the Phaseolus enzyme has but little action on amygdalin. The existence of this enzyme was not yet recognised at the time when Dunstan, Henry and Auld were led to conclude that the Phaseolus glucoside is an α-derivative.*

Although the experiments we have made with heated and unheated extracts from various yeasts have given very irregular results, it appears to us that they afford conclusive evidence that phaseolunatase is not amygdalase. As often as not, little or no action was noticeable, whilst in some cases 5 or 6 per cent. at most of the Phaseolus glucoside was hydrolysed within 24 hours at 25°: any action observed in such cases we are inclined, therefore, to attribute to the presence of a minute proportion of "emulsin."

In preparing Fischer's glucoside from amygdalin by means of yeast, some hydrogen cyanide is always liberated, the yield of the glucoside being by no means satisfactory.

On one occasion an exceptionally strong extract prepared by digesting 20 grammes of a particular fresh yeast with 100 c.c. of water was found to

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* Auld has made the assumption that amygdalase is an α-enzyme and has described experiments which he assumes afford proof that the glucose liberated when amygdalin is resolved into Fischer's glucoside and glucose is the α-form and that liberated from Fischer's glucoside the β-form. In our opinion, his argument is rendered invalid by the fact that both amygdalin and Fischer's glucoside are "racemised" somewhat rapidly by alkali; E. F. Armstrong's method, therefore, cannot well be applied to these materials.

The following observations are quoted as showing the extent to which change takes place. A solution of mandelonitrile-glucoside contained in a 2-decimetre jacketed polarimeter tube kept at 25° having been mixed with a drop of 3N ammonia, observations of the optical rotation were taken at minute intervals.

	°
Before the addition of alkali the rotation was........................	−1·77
1 min. after the addition of alkali the rotation was................	−1·85
2 mins. „ „ „ „	−1·93
3 „ „ „ „ „	−2·03
4 „ „ „ „ „	−2·12
5 „ „ „ „ „	−2·21
6 „ „ „ „ „	−2·27
7 „ „ „ „ „	−2·35
8 „ „ „ „ „	−2·42
9 „ „ „ „ „	−2·47
10 „ „ „ „ „	−2·53
30 „ „ „ „ „	−3·23

Three comparative experiments were also made by taking equivalent solutions of amygdalin, mandelonitrile-glucoside and ordinary (*a*) glucose; after observing the optical rotatory power, each was mixed with the same proportion of alkali (one drop of 3N ammonia) and the change of rotation determined when all three solutions were kept effect the decomposition of no less than 13·5 per cent. of the glucoside; although very active towards methyl-α-glucoside, this extract had no action on amygdalin, a quite unusual circumstance. On repeating the experiment several months later with the same yeast but with an extract prepared by digesting only 15 grammes with 300 c.c. of water, no action was observed either on phaseolunatin or on amygdalin.

The probability that yeast contains a "β-glucase" is considerable in view of the fact that cellulose is now known to be a compound of the β-glucoside class; cytase (cellase), if not "β-glucase," is probably a closely allied enzyme.

It is clear, however, that not only do different yeasts yield different enzymes or different proportions of the several enzymes, but also that age, as well as race and the conditions under which the yeast is dried and extracted, are all factors to be taken into account as influencing the character of the extract.

Nature of the Dextrose Residue in Phaseolunatin.—Dunstan, Henry and Auld give the following description of their experiments made with the object of characterising the glucose produced by the hydrolysis of phaseolunatin :—

"To a solution of 3 grammes of the glucoside in 20 c.c. of water, 0·5 gramme of the enzyme preparation was added and the mixture kept at 40° in a stoppered bottle. For each observation a few cubic centimetres of the liquid were withdrawn, mixed with a small

under observation at the ordinary temperature (15°). The following are the results obtained :—

	Glucose.	Mandelonitrile glucoside.	Amygdalin.
	°	°	°
Initial rotation	+3·89	−1·91	−3·83
Rotation 1 minute after adding alkali.........	+3·57	−1·93	−3·84
,, 2 minutes ,, ,, ...	+3·32	—	−3·88
,, 3 ,, ,, ,, ...	+3·06	−2·03	−3·91
,, 4 ,, ,, ,, ...	+2·89	−2·10	−3·94
,, 5 ,, ,, ,, ...	+2·73	−2·15	−3·95
,, 6 ,, ,, ,, ...	+2·63	−2·18	−3·98
,, 7 ,, ,, ,, ...	+2·53	−2·21	−4·02
,, 8 ,, ,, ,, ...	+2·48	−2·22	−4·05
,, 9 ,, ,, ,, ...	+2·37	−2·25	−4·06
,, 10 ,, ,, ,, ...	+2·33	−2·31	−4·08

From these figures, it is obvious that the change undergone by glucose is more rapid than that undergone by mandelonitrile-glucoside and by amygdalin; nevertheless, the extent to which racemisation of the latter two substances takes place in the time (according to Lowry about 12 minutes in the presence of N/100 alkali such as we used) required to effect the complete muta-rotation of glucose is too great to permit the application of E. F. Armstrong's method in the presence of the two cyanophoric glucosides.

definite quantity of alumina cream and immediately filtered through a closely-felted pad of asbestos. The optical rotation of the clear filtrate was then determined *before* and *after* addition of a drop of ammonia solution, the latter being used, as indicated by Lowry, to establish equilibrium. The same polarimeter tube was used throughout the experiments."

Time of action.	Initial rotation.	Rotation after addition of ammonia.	Change in rotation.
Hours.	° ′	° ′	° ′
0	−1 15	−1 15	0 0
1	−0 46	−1 11	−0 25
2	−0 35	−0 58	−0 23
3	−0 34	−0 57	−0 23
20	−0 56	−0 56	0 0

With reference to these figures, it may be noted that a solution of 3 grammes of phaseolunatin in 20 c.c. of water will have an optical rotatory power of −4°·11 (approximately); in order to reduce this to the observed −1°·25, the solution must have been treated with almost three times its volume of alumina cream, supposing the observations were made in a 1-decimetre tube; such an amount can scarcely be described as "small."

Secondly, if we are to assume that the same proportion of alumina cream was always used—and otherwise we do not understand the meaning of the word "definite"—the changes in rotation given in the fourth column above are far larger than can possibly have been produced by the muta-rotation of glucose. The decomposition of the phaseolunatin contained in a 50-per-cent. solution (the optical rotation of which in a 2-decimetre tube is identical with the specific rotatory power of the glucoside) into hydrogen cyanide, acetone and α-glucose would involve a change in the rotatory power of 104°, 27°·4 being consequent on the destruction of the glucoside and 76°·6 on the liberation of α-glucose. Assuming that the glucose set free does not change spontaneously, any decrease in the lævo-rotatory power of a solution of phaseolunatin which is undergoing hydrolysis will be one-fourth due (27·4/104 exactly) to the disappearance of glucoside and three-fourths to the liberation of α-glucose. The initial rotation is given as −1° 15′ and that after one hour as −0° 46′, thus showing a fall in lævo-rotation of 29′. Of the latter, as indicated above, only three-fourths or approximately 21′ can be due to the production of α-glucose. The muta-rotation of glucose causes a change in the specific rotatory power from 105° to 52°·7, that is to say roughly a decrease of 50 per cent.; hence the fall of 21′, due to the liberation of α-glucose, will be reduced to 10′·5 on adding alkali, that is to say to a change of 10′·5 (roughly), whereas that observed was 25′. The same argument can be applied to the other values given.

On reference to E. F. Armstrong's account of his work* it will be found that his original observations are, in most cases, affected by similar "errors." It would seem that the changes taking place in the solutions examined are not only those which are involved in the alteration of the glucose originally produced into the equilibrated mixture of isomerides—in fact, the substances present in the solution of the enzyme are also affected by the alkali.

Our experiments have afforded abundant evidence that such is the case, as will be pointed out later on. Moreover, we find that the Phaseolus enzyme is itself sensitive to alkali; thus a solution of which the initial rotatory power was +0°·01 was found to be considerably altered, the rotatory power changing from −0°·02, the value after adding alkali, to −0°·95 at the end of four hours.

Under these circumstances we have deemed it desirable to repeat some of E. F. Armstrong's experiments, in order to verify his statements—especially as the instrumental appliances at our disposal are far better than those over which he had command. The following series of observations made with methyl-β-glucoside and emulsin are a complete justification of his method:—

Time of action.	Initial rotation.	Rotation after addition of ammonia.	Change in rotation.
hours.	°	°	°
0	−2·83	−2·80	+0·03
1	−2·30	−2·14	+0·16
1·5	−2·03	−1·85	+0·18
2	−1·74	−1·59	+0·15
2·5	−1·53	−1·38	+0·15
4	−0·71	−0·61	+0·10
4·5	−0·54	−0·44	+0·10
6	−0·12	−0·04	+0·08
24	+2·63	+2·62	−0·01

Attempts to carry out a similar experiment with maltose were unsuccessful owing to our failure to obtain enzyme extracts which remained clear on the addition of alkali. It may be mentioned that in E. F. Armstrong's experiments the liquid was filtered to remove the turbidity occasioned by alkali; we have sought to avoid any such operation and have added the alkali directly to the liquid in the tube.

An M/5 phaseolunatin solution containing 25 c.c. of phaseolunatase solution per 100 c.c. was kept in a Jena flask at 37°. At hourly intervals, 20-c.c. samples were withdrawn, mixed with 5 c.c. of alumina cream, rapidly filtered through asbestos and examined polarimetrically at 25° in a 2-decimetre tube. Then two drops of soda solution were added and the rotation again determined.

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* 'Chem. Soc. Trans.,' 1903, vol. 83, p. 1305.

Time of action.	Initial rotation.	Rotation after addition of soda.	Change in rotation.
hours.	°	°	°
0	−3·40	−3·36	+0·04
1	−3·12	−3·11	+0·01
2	−2·83	−2·80	+0·03
3	−2·57	−2·55	+0·02
3·5	−2·43	−2·43	0·0
5	−2·10	−2·11	−0·01
6	−1·80	−1·81	−0·01
7	−1·65	−1·65	0·0
8	−1·51	−1·50	+0·01
25	−0·27	−0·21	+0·06

The differences observed in this case were too small to be of any positive value. Further experiments were carried out as follows:—

A solution of 7·4143 grammes of phaseolunatin in water occupying 75 c.c. at 25° was mixed with 75 c.c. of a solution of phaseolunatase, also at 25°. The latter solution was prepared by shaking 100 c.c. of the cloudy extract with 20 c.c. of alumina cream and filtering; this treatment gave a clear solution of the enzyme, highly active towards phaseolunatin. A 2-decimetre jacketed polarimeter tube was filled with the mixture of glucoside and enzyme and the rotation observed at 25°; then a drop of a solution of soda was added and the rotation again determined. Meanwhile the main bulk of the liquid (which was now an M/5-solution of the glucoside) was heated to 37° and kept at this temperature, samples being withdrawn at intervals and examined in the manner described in the case of the initial experiment. The following results were obtained:—

	Time of action.	Initial rotation.	Rotation after addition of soda.	Mean change in rotation.
	Hours.	°	°	°
1st experiment...	0	−4·24	−4·21	+0·03
	1	−3·99	−3·95	+0·04
	2	−3·57	−3·51	+0·06
	3	−3·26	−3·23	+0·03
	4·5	−2·81	−2·81	0·0
	5	−2·56	−2·55	+0·01
	6	−2·31	−2·31	0·0
	7	−2·04	−2·11	−0·07
	24	−0·27	−0·36	−0·09
2nd experiment...	0	−3·76	−3·74	+0·02
	1	−3·66	−3·65	+0·01
	1·5	−3·53	−3·50	+0·03
	2·5	−3·27	−3·26	+0·01
	4	−2·90	−2·90	0·0
	5·5	−2·61	−2·66	−0·05
	6·5	−2·45	−2·49	−0·04
	24	−0·35	−0·44	−0·09

We are of opinion that the change indicated in these various cases is in the direction corresponding with the conversion from β- into α-glucose, not from α into β. But this change is complicated by some change in the other substances present in the solution which somewhat masks that undergone by the glucose.

The complete set of readings obtained in the course of another experiment may be given with advantage, as illustrating the procedure and also because of the evidence they afford that changes are proceeding such as have been referred to. The equilibration of the glucose by means of the alkali added (1 drop of a 10 N solution) is practically an instantaneous process. It should be mentioned that the set of readings given on each of the lines in the table would occupy about five to six minutes, readings being taken at intervals of about half a minute.

Time.	Polarimeter readings.	Mean rotation.	Change in rotation.
1 hour	Initial −4°·20, 21, 23, 23, 20, 22, 23, 20, 23, 22	−4°·22	+0°·05
	+alkali −4°·18, 20, 17, 21, 14, 13, 18, 18, 17, 14	−4°·17	
1·5 hours ...	Initial −3°·96, 97, 96, 95, 95, 94, 95, 96, 94, 95	−3°·95	+0°·04
	+alkali −3°·91, 90, 94, 94, 93, 93, 89, 89, 94, 89	−3°·91	
2 " ...	Initial −3°·69, 69, 69, 67, 70, 66, 68, 69, 68, 68	−3°·68	+0°·01
	+alkali −3°·64, 64, 68, 69, 67, 67, 67, 67, 67, 67	−3°·67	
3·5 " ...	Initial −2°·77, 78, 79, 78, 79, 82, 83, 80, 81, 81	−2°·80	−0°·04
	+alkali −2°·77, 80, 82, 87, 84, 85, 87, 89, 85, 89	−2°·84	
4 " ...	Initial −2°·59, 55, 60, 55, 57, 58, 58, 57, 57, 58	−2°·57	−0°·06
	+alkali −2°·60, 60, 60, 58, 61, 63, 67, 66, 67, 70	−2°·63	
5 " ...	Initial −2°·08, 07, 08, 11, 10, 11, 10, 08, 10, 11	−2°·09	−0°·09
	+alkali −2°·07, 13, 08, 24, 24, 18, 20, 24, 21, 23	−2°·18	
6 " ...	Initial −1°·44, 43, 44, 43, 46, 46, 45, 45, 47, 46	−1°·45	−0°·09
	+alkali −1°·48, 52, 52, 54, 53, 54, 57, 58, 55, 55	−1°·54	
24 " ...	Initial +0°·56, 54, 56, 55, 54, 55, 56, 56, 55, 55	+0°·55	−0°·14
	+alkali +0°·48, 40, 42, 42, 41, 40, 42, 40, 41, 40	+0°·41	

The figures given in the last column are obtained from the mean rotations before and after the addition of alkali. If, however, the first reading after the addition of alkali be compared with the last prior to the addition—thus reducing the possible change of the enzyme to a minimum—the changes in rotation become +0°·04, +0°·04, +0°·04, +0°·04, −0°·02, +0°·04, −0°·02, and −0°·07 respectively, values which lend still more support to the conclusion that the glucose is liberated in the β-form. Moreover, the observation in all three experiments of a comparatively large change produced by adding alkali to the 24-hour sample, when presumably all the glucose is present as an equilibrated mixture, is evidence that the enzyme suffers a change (in optical rotatory power) in a direction contrary to that undergone by the liberated glucose.

Comparative Action of Emulsin and of the Phaseolus Enzyme on Fischer's Glucoside, Amygdalin and the Phaseolus Glucoside.—Assuming that the resolution of amygdalin is primarily conditioned by amygdalase and that any β-glucase which it contains cannot operate until the diglucoside is resolved into Fischer's glucoside and glucose, the inactivity of the Phaseolus enzyme towards amygdalin may be ascribed to the absence from it of all but traces of amygdalase. From this point of view, Fischer's glucoside, salicin and methyl-β-glucoside, rather than amygdalin, are to be compared with the Phaseolus glucoside and are the appropriate test materials to use in determining whether the correlated enzyme belongs to the β class, whether indeed it may not be simple β-glucase but present in considerably smaller proportion than in emulsin.

Our observations show that methyl-β-glucoside is far less readily attacked by the Phaseolus enzyme than it is by almond-emulsin. It is well known that this glucoside is somewhat rapidly attacked by emulsin; that phaseolunatase acts on it only slowly is shown by the following figures derived from an experiment made with an M/5 solution containing 50 c.c. of the enzyme extract per 100 c.c. :—

Time of action............	1 day.	2 days.	7 days.	9 days.	15 days.	17 days.	33 days.
Percentage of hydrolysis	2·3	7·2	26·1	30·5	40·6	43·7	51·5

In the case of salicin, on the other hand, the disproportion in the rates of change appears to be far less marked.

To ascertain the behaviour of the three cyanophoric glucosides, experiments were made as follows: six solutions were prepared:—

(1) By mixing 15 c.c. of a 4M/10 amygdalin solution with 15 c.c. of a dilute solution (1 : 10) of emulsin.

(2) By mixing 15 c.c. of a 4M/10 amygdalin solution with 15 c.c. of the solution of phaseolunatase.

(3) By mixing 15 c.c. of a 4M/10 Fischer's glucoside solution with 15 c.c. of the dilute solution of emulsin.

(4) By mixing 15 c.c. of a 4M/10 Fischer's glucoside solution with 15 c.c. of the phaseolunatase solution.

(5) By mixing 15 c.c. of a 4M/10 solution of phaseolunatin with 15 c.c. of a strong solution of emulsin.

(6) By mixing 15 c.c. of a 4M/10 solution of phaseolunatin with 15 c.c. of phaseolunatase solution.

These were kept at 37°. The same solution of enzyme was used in the case of all three glucosides, except that the emulsin used with phaseolunatin was ten times as strong as that used in the other cases. After 24 hours the amount of hydrolysis effected was determined in each case by estimating the amount of hydrogen cyanide liberated.

Hydrolyst.	Solution (30 c.c.) containing					
	3·0676 grammes amygdalin.		1·7709 grammes Fischer's glucoside.		1·4828 grammes phaseolunatin.	
	Weight hydrolysed.	Percentage hydrolysed.	Weight hydrolysed.	Percentage hydrolysed.	Weight hydrolysed.	Percentage hydrolysed.
Emulsin, 5 hours	2·162	70·5	1·655	93·5	—	—
Emulsin, 24 hours	2·5	81·5	1·595	*89·5	0·052	3·5
Phaseolunatase, 24 hours	0·107	3·5	1·533	86·0	1·334	90·0

* The lower percentage in this instance is to be accounted for by loss of hydrogen cyanide on removal of the 5-hour sample. Whilst no appreciable loss occurs at 25° through the removal of samples at intervals, this is not the case at the higher temperature.

The striking fact brought out by these experiments is the manner in which the Phaseolus enzyme acts equally well on Fischer's glucoside and phaseolunatin, although it has but little action on amygdalin. On the assumption that emulsin is relatively rich in β-glucase—far more so than the Phaseolus enzyme—and that both glucosides are of the β-type because of this similar behaviour, it is difficult to understand the inactivity of emulsin towards phaseolunatin. It is to be noted that our observations do not stand alone: Dunstan and his co-workers came to the conclusion that emulsin has no action on phaseolunatin.

It would seem that there are peculiarities either in the enzymes or in the glucosides which await discovery. That the Phaseolus enzyme should not act on amygdalin is not surprising, as it may well be destitute of amygdalase; it is, however, difficult to explain the inactivity of almond-emulsin towards phaseolunatin, in view of the activity of phaseolunatase towards Fischer's glucoside as well as towards phaseolunatin; such a result is scarcely in favour of the view that the activity of emulsin as a hydrolyst of β-glucosides is due to a simple β-glucase, *i.e.* an enzyme compatible with and influenced by glucose alone.

As bearing on this issue, the following experiments were made with pressed yeast juice kindly placed at our disposal by Dr. Harden: four solutions were prepared:—

(1) (3) By mixing 10 c.c. of a 4M/10 solution of Fischer's glucoside with 10 c.c. of the yeast juice.

(2) (4) By mixing 10 c.c. of a 4M/10 solution of phaseolunatin with 10 c.c. of the pressed yeast juice.

Solutions (1) and (2) were kept at 37°, (3) and (4) at 25°. The yeast juice was used within 30 minutes of receipt, *i.e.* within an hour of leaving Dr. Harden's hands. After 23 hours the extent to which hydrolysis had taken place in each case was measured by estimating the hydrogen cyanide liberated.

Temperature.	Solution (20 c.c.) containing			
	1·1806 grammes Fischer's glucoside.		0·9886 gramme phaseolunatin.	
	Weight hydrolysed.	Percentage hydrolysed.	Weight hydrolysed.	Percentage hydrolysed.
° 25	0·0915	7·75	0·0049	0·5
37	0·1682	14·25	0·0098	1·0

Seven days later, two experiments were made (at 37°) to ascertain whether the yeast juice had retained its activity and also to contrast its behaviour towards Fischer's glucoside with that towards amygdalin; the results are remarkable as showing comparatively slight activity towards the latter, only 5 per cent. of amygdalin but 13·5 per cent. of the Fischer's glucoside being changed. The yeast enzyme appears to resemble almond-emulsin in its behaviour towards the Phaseolus glucoside.

Rosenthaler's observations, as already pointed out, serve to raise the question whether there may not be in almond-emulsin an enzyme or enzymes capable of affecting the cyanophoric radicle in amygdalin. (He suggests that both a synthetic and an analytic enzyme are present.)

The fact that mandelonitrile may be separated as such from amygdalin by the action of sulphuric acid of a certain degree of concentration* would appear to lend support to this contention; this and other similar nitriles have been shown by Ultée† to be far more stable compounds than was formerly supposed, so that the production of hydrogen cyanide from amygdalin is not to be set down to the mere spontaneous decomposition of the nitrile when liberated in aqueous solution.

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* Walker and Krieble, 'Chem. Soc. Trans.,' vol. 95, 1909, p. 1374.

† 'Rec. Trav. Chim.,' 1909, vol. 28, pp. 1—23; 248—260.

From this point of view, it is possible that a distinct enzyme—an *acetcyanase*—is operative in separating the nitrilic radicle from phaseolunatin and that this, rather than a simple β-glucase, is the main constituent of the active product from Phaseolus beans; but if such be the case, the enzyme must be compatible with the cyanophoric radicle in Fischer's glucoside, whilst the corresponding enzyme in emulsin must be incompatible with the nitrilic member of phaseolunatin.

Although we have been able to verify Rosenthaler's statements as to the synthetic activity of almond-emulsin, we have been unsuccessful in preliminary experiments with phaseolunatase in obtaining evidence that this enzyme can induce the interaction of benzaldehyde and hydrogen cyanide. This result apparently affords additional proof that the almond and the Phaseolus enzymes are possessed of distinctive properties. The problem afforded by "emulsin" is one which can only be solved by exhaustive study of enzymes from various sources. In conjunction with Dr. E. F. Armstrong, we have for some time been engaged in developing a method of standardising the enzymes relatively to a series of reference compounds, so that a strict comparison may be made of the activities of materials from various sources.

[To contrast the behaviour of vegetable *cytase*—which may be regarded provisionally as identical with *cellase*, the enzyme by which the β-glucoside *cellobiose* is resolved into two molecules of glucose*—with that of almond emulsin and of the Phaseolus enzyme, we have ascertained the effect produced on several cyanophoric glucosides by an extract prepared by digesting ground oats with three times their weight of water during about 15 hours. A mixture of the extract with an equal volume of a 4M/10 solution of the glucoside was digested at 37° during 48 hours. The amount of change was 8 per cent. in the case of Fischer's glucoside, 5 per cent. in that of amygdalin and 4 per cent. in the case of the Phaseolus glucoside. The greater sensitiveness of this last in comparison with amygdalin, when the results are compared with those produced by emulsin, is perhaps noteworthy and suggestive.

We may also refer to results obtained with an extract of the seeds of the kidney vetch, *Anthyllis vulneraria*, which contains an enzyme of the emulsin type. To test its activity the ground seed was macerated with about four times its weight of water. An M/5 solution of the glucoside containing half its bulk of this extract was digested at 37° during 24 hours. The amount of change was 13·5 per cent. in the case of Fischer's glucoside but only 3 per cent. in that of amygdalin and 2 per cent. in that of the Phaseolus glucoside.

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* *Cf.* E. Fischer and G. Zemplén, 'Annalen,' 1909, vol. 365, pp. 1–6; Bertrand and Holderer, 'Compt. Rend.,' 1910, vol. 150, p. 230; Pringsheim and Zemplén, 'Zeit. Physiol. Chem.,' 1909, vol. 62, p. 367.

It can scarcely be doubted that several specific enzymes exist which may all be spoken of as *β-glucases.* The common factor in these presumably is a *primary member* sympathetic with the glucose member of the glucoside; the *secondary member*—in the case of the cyanophoric glucosides, the group CXY(CN)—being different in the several enzymes. We are led to infer the existence of such a primary member sympathetic with glucose in the case of almond-emulsin and in that of the Phaseolus enzyme, as these are both controlled to a marked extent by glucose, less action taking place if glucose be added in advance.

On such an assumption, it is not difficult to understand the inactivity, for example, of the Phaseolus enzyme towards amygdalin as compared with Fischer's glucoside. It may be supposed that the molecule of glucose attached to the Fischer's glucoside in amygdalin is so placed as to render association between the hydrolyst and the primary member of the hydrolyte impossible or nearly so, not only in the case of the Phaseolus enzyme but also in that of almond-emulsin, action taking place in the case of the latter only after the amygdalase, which accompanies the β-glucase, has prepared the way by removing the terminal glucose group.—March 10, 1910.]

[The 'Comptes Rendus' of March 21 (vol. 150, pp. 793—796) contains the welcome announcement by J. Giaja that by means of an extract prepared from the snail *Helix pomatia*, L., he has succeeded in separating from amygdalin a non-reducing saccharose of the trehalose type. April 9.]

[The cost of this investigation has been in large part met by a grant, for which I am indebted to the Government Grant Fund of the Royal Society.—H. E. A.]

The Development of Trypanosomes in Tsetse Flies.

By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service.
(Sleeping Sickness Commission of the Royal Society, 1908–10.)

(Received April 18,—Read May 5, 1910.)

In the 'Proceedings' of the Royal Society (B, vol. 81, 1909) a paper was published describing a single experiment illustrating the development of *Trypanosoma gambiense* in *Glossina palpalis.* This experiment was carried out at Mpumu, Uganda, near Lake Victoria, in the spring of 1909. Since that date many experiments, on the same lines, have been made, not only with *Trypanosoma gambiense* but also with *Trypanosoma dimorphon, Trypanosoma nanum,* and *Trypanosoma vivax.** It is proposed to describe these further experiments in this paper.

It will be remembered that Kleine, in German East Africa, at the end of 1908, made the discovery that *Glossina palpalis* could convey *Trypanosoma brucei* for some 50 days after the fly had fed on an infected animal. Following Kleine's lead, our experiments were carried out, at first with Lake-shore flies, afterwards with flies bred in the laboratory.

A. *The Development of* Trypanosoma gambiense *in* Glossina palpalis *caught on the Lake-shore.*

These experiments were carried out with ordinary wild tsetse flies caught on the Lake-shore, and therefore open to the doubt that some of them may have been naturally infected when they were captured. As there is some evidence that one fly in 400 or 500 of the wild Lake-shore flies is found to be naturally infected, it is evident that these previously infected flies may lead into error. It will be seen later that this risk is not run when flies bred in the laboratory are used.

The flies when brought up from the Lake-shore were kept in small boxes, with mosquito-netting sides, and placed over dishes containing water, to imitate, as far as possible, their natural conditions. It may be remarked here that these tsetse flies are so numerous on the shores of Victoria Nyanza, and the supply so unending, that the fly-boys brought up some 500 every day, and these usually caught at only one or two spots.

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* These names may require to be changed, when the trypanosomes affecting domestic animals in Uganda come to be described.

The method of carrying out these experiments was always the same. The flies were fed for some days on a highly infected monkey, whose blood on microscopical examination was seen to contain numerous trypanosomes of Sleeping Sickness, and afterwards on a series of healthy monkeys.

The following table gives the number of flies used in each experiment, the number of days they were fed on a monkey whose blood contained *Trypanosoma gambiense*, the number of days which elapsed before the flies became infective, and the number of days the flies remained infective. The minus signs signify that the flies failed to become infected, or at least failed to infect; or, in other words, that the experiment was negative.

Table I.—Development of *Trypanosoma gambiense* in Lake-shore *Glossina palpalis.*

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
624	60	3	—	—
656	280	3	—	—
663	60	2	18	75
676	500	3	29	47
721	50	3	—	—
980	350	3	—	—
986	100	2	19	35
987	50	2	40	51
989	50	2	45	45
1020	100	3	—	—
1023	50	3	37	51
1026	70	3	34	48
1198	20	40	—	—
1372	100	3	—	—

Of these 14 experiments, seven are positive and seven negative. In the positive experiments 880 flies were used, an average of 126; in the negative 960, an average of 137. The shortest time which elapsed before a fly became infective was 18 days, the longest 45 days, and the average 32 days.

It may be well to give some of these experiments more in detail, in order to show the methods used, and draw attention to various interesting points.

Experiment 624.

To ascertain if development of *Trypanosoma gambiense* takes place in the alimentary canal of Wild or Lake-shore *Glossina palpalis.*

March 31, 1909.—Two batches of *Glossina palpalis*, caught on the Lake-shore, consisting of 30 flies in each batch, were fed to-day on a monkey whose blood contained numbers of *Trypanosoma gambiense.*

The following table gives the principal details of the experiment :—

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Mar. 31	—	Flies fed on infected monkey.			
Apr. 1	1	" "			
" 2	2	" "			
" 3—4	3—4	Flies starved 72 hours.			
" 5—8	5—8	Flies fed on healthy monkey, 657		—	
" 9—12	9—12	Flies fed on healthy monkey, 677		—	
" 13	13	Flies fed on healthy monkey, 702		—	
" 14	14	Flies fed on healthy monkey, 703		—	
" 15	15	Flies fed on healthy monkey, 704		—	
" 16	16	Flies fed on healthy monkey, 705		—	
" 17—21	17—21	Flies fed on healthy monkey, 706		—	
" 22—May 22	22—52	Flies fed on healthy monkey, 728		—	

Remarks.—These 60 Lake-shore or wild flies, although fed on an infected monkey for three days, failed to convey the infection to healthy monkeys. As the flies died they were dissected. Only in one were flagellates found, and these appeared to be of the *Trypanosoma grayi* type. An emulsion was made of the contents of the alimentary canal of this fly and injected into monkey 914. Monkey 914 never showed trypanosomes in its blood, although kept under observation for a month.

Experiment 656.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Apr. 3—5	1—2	280 flies fed on infected monkey.			
" 6—7	3—4	Flies starved 72 hours.			
" 8—17	5—14	Flies fed on healthy monkey, 675		—	
" 18	15	Flies fed on healthy monkey, 712		—	
" 19	16	Flies fed on healthy monkey, 713		—	
" 20	17	Flies fed on healthy monkey, 714		—	
" 21	18	Flies fed on healthy monkey, 715		—	
" 22—25	19—22	Flies fed on healthy monkey, 716		—	
" 26—28	23—25	Flies starved.			
" 29—June 17	26—75	Flies fed on healthy monkey, 744		—	June 8, 8 flies alive. June 18, 2 flies alive.

Remarks.—Two hundred and eighty wild tsetse flies, fed for three days on an infected monkey and then on healthy monkeys, failed to transfer the disease. The experiment lasted from April 3 to June 17, and seven healthy monkeys were used. After 66 days eight flies remained alive ; after 75 days only two. None of the flies which died, or were killed and dissected, showed flagellates in the alimentary canal.

Experiment 663.

This is the experiment described at length in the 'Proceedings' (B, vol. 81, 1909). Sixty wild flies were used. One fly became infective after 18 days, and remained infective 75 days, when it died. A small quantity of fluid from the gut of this fly injected into a healthy monkey gave rise to Sleeping Sickness.

Experiment 676.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Apr. 6—8	1—2	500 Lake-shore flies fed on infected monkey.			
,, 9—10	3—4	Flies starved.			
,, 11—16	5—10	Flies fed on healthy monkey, 696		—	
,, 17—18	11—12	Flies fed on healthy monkey, 707		—	
,, 19—20	13—14	Flies fed on healthy monkey, 708		—	
,, 21	15	Flies fed on healthy monkey, 709		—	
,, 22	16	Flies fed on healthy monkey, 710		—	
,, 23—May 12	17—36	Flies fed on healthy monkey, 711	+		April 23, 67 flies alive
May 13—14	37—38	Flies starved.			
,, 15—16	39—40	Flies fed on healthy monkey, 766	+		
,, 17—21	41—45	Flies fed on healthy monkey, 770	+		
,, 22—23	46—47	Flies starved ....,			May 23, 22 flies alive. Infected fly found.
,, 24—28	48—52	Flies fed on healthy monkey, 901		—	
,, 29—30	53—54	Flies starved.			
,, 31—June 3	55—58	Flies fed on healthy monkey, 941		—	June 3, 1 fly alive.

Remarks.—Five hundred Lake-shore tsetse flies were fed for three days on an infected monkey. As none of the five healthy monkeys on which these flies were fed during the first 16 days showed any sign of Sleeping Sickness it may be assumed that there was no naturally-infected fly among the 500. On or about the 29th day the cage of flies became infective, and remained infective up to the 47th day. On this day a dead fly was found on dissection to contain flagellates, and after the death of this fly no further infection took place. The injection of the infected fly failed, however, to give rise to Sleeping Sickness when injected under the skin of a healthy monkey.

Experiment 721.

Remarks.—This experiment lasted from April 12 to July 6, a period of 85 days, and remained negative throughout. On the 30th day, 12 flies out of the 50 remained alive; on the 73rd day only six flies were left. As the flies died they were dissected, but no infected fly was found.

Experiment 980.

Remarks.—This experiment lasted 66 days, and remained negative. At the end 15 flies remained alive. These were killed and dissected. All proved negative.

These experiments on the development of *Trypanosoma gambiense* in Lake-shore or wild *Glossina palpalis*, given somewhat in detail, will suffice to show the method employed, and make it unnecessary to explain the remaining experiments further than is done in Table I.

It would appear from the fact that none of the healthy monkeys became infected before the 18th day, that not a single fly of the 1840 used was infective when captured. That is to say, that among nearly 2000 Lake-shore flies, not one was naturally infected. On referring to Table IV, in the previous paper in the 'Proceedings'—a table showing the probable number of naturally-infected flies—this is seen to be by no means exceptional.

Other points of interest arising out of these experiments are the number of flies which became infective, and the result of injecting their body-contents into healthy animals.

The following table shows this:—

Table II.—Number of Flies found Infected with Trypanosomes in the Experiments with Lake-shore Flies and *Trypanosoma gambiense*.

Experiment.	No. of flies used.	Experiment, positive or negative.	No. of infected flies found.	Result of injection of infected flies.	Remarks.
624	60	–	1	Negative	*T. grayi* type.
656	280	–	0		
663	60	+	1	Positive.	
676	500	+	1	Negative.	
721	50	–	0		
980	350	–	0		
986	100	+	1	Not injected.	
987	50	+	2	Negative	1 *T. gambiense* and 1 *T. grayi* type.
989	50	+	1	Negative.	
1020	100	–	0		
1023	50	+	5	2 positive. 3 negative.	
1026	70	+	4	3 positive. 1 negative.	
1198	20	–	0		
1372	100	–	0		

Thus it is seen that the infected flies found in three of the positive experiments when injected into healthy monkeys gave negative results, while those found in three others gave positive results. The infected fly found in the seventh successful experiment was not injected.

It is difficult to understand why the results of injecting infected flies into healthy animals are so irregular. The only theory brought forward is that the trypanosomes introduced under the skin along with the tissues of the fly may give rise to a reaction at that point, which will so damage the parasites that they fail to infect.

In these experiments, 1840 flies were used, and of these 16 became infected, or, at least, were found to have flagellates in their gut. This works out at less than 1 per cent. The smallness of the percentage is due to the fact that less care was taken to dissect the flies which died during the course of the experiments.

B. *The Development of* Trypanosoma gambiense *in Laboratory-Bred* Glossina palpalis.

The pupæ of the fly were found on the Lake-shore, and hatched out in the laboratory. For a long time the Commission failed to find any pupæ, although days were spent in turning over soil and decaying vegetable matter in those places where the fly most abounded. At last, Lieutenant A. D. Fraser, Royal Army Medical Corps, found them in numbers in patches of sand on the edge of the Lake in the Sesse Islands. After the Sesse Islands were emptied of their inhabitants, Fraser's native collectors came into the service of the Commission, and from that time there was no lack of pupæ. These natives found them in large numbers. One day they brought up as many as 7000. These pupæ proved to be much healthier than those obtained from flies in captivity. The flies bred from larvæ born in the laboratory rarely showed any marked vitality. Many of the larvæ were immature, and those which hatched out were rarely a success as experimental flies. On the other hand, the flies hatched out from pupæ found on the Lake-shore were fairly strong and vigorous, and lived in captivity for a couple of months or more. It was, however, difficult to get them to feed at first, and very few became infective, as the following table shows. The flies were fed chiefly on infected monkeys. In one negative experiment (1431) they were fed on a case of Sleeping Sickness in man, and in five—two positive (1566 and 1602) and three negative (1269, 1452, and 1672)—on oxen. Numerous observations went to show that there is no hereditary transmission of trypanosomes in *Glossina palpalis*; and no evidence was gained that the flies became infected with any flagellate by contact with other flies or fouled cages. Any trypanosomes found in laboratory-bred flies may therefore be considered to be derived from the infected animal they had fed upon.

Table III.—Development of *Trypanosoma gambiense* in Laboratory-Bred *Glossina palpalis.*

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
725	8	3	—	—
733	8	3	—	—
750	15	6	—	—
768	9	5	—	—
858	9	8	—	—
915	6	10	—	—
947	9	5	—	—
975	16	5	34	49
991	27	6	—	—
1266	11	12	—	—
1269	22	7	—	—
1368	14	12	—	—
1397	7	19	—	—
1428	50	6	—	—
1431	30	20	—	—
1452	90	10	—	—
1549	50	4	37	46
1558	60	4	—	—
1559	50	4	—	—
1566	35	4	53	53
1602	50	4	27	27
1604	35	12	—	—
1640	40	10	—	—
1651	60	4	41	41
1664	50	12	—	—
1665	50	12	—	—
1671	45	12	—	—
1672	28	12	—	—
1680	45	4	—	—
1686	60	3	—	—
1688	60	3	—	—
1693	50	4	—	—
1706	60	7	—	—
1712	50	12	32	32
1758	50	3	—	—
1760	60	4	29	29
1761	50	4	38	38
1769	60	3	—	—
1801	70	12	—	—
1802	75	12	—	—
1860	60	12	—	—
1868	60	12	—	—

Among these 42 experiments there were only eight positive, while there are as many as 34 negative. In the eight positive experiments, 371 flies were used, an average of 46; in the 34 negative experiments, 1323 flies, an average of 40. The shortest time which elapsed before a laboratory-bred fly became infected with *Trypanosoma gambiense* was 27 days, the longest 53 days, and the average 36 days.

Here follow the experiments, given somewhat more in detail, which gave positive results:—

Experiment 975.

To ascertain if any development of *Trypanosoma gambiense* takes place in the alimentary canal of laboratory-bred *Glossina palpalis*.

The following table gives the principal details of the experiment:—

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. June 8—12	1—4	Flies fed on infected monkey			16 flies used.
„ 13—15	5—7	Flies starved.			
„ 16—July 19	8—41	Flies fed on healthy monkey, 1008	+		
July 20—21	42—43	Flies starved.			Aug. 4, 12 remaining flies killed and dissected.
„ 22—Aug. 3	44—55	Flies fed on healthy monkey, 1374	+		

Remarks.—Sixteen laboratory-bred flies were fed on infected monkeys for five days. Thirty-four days after their first feed they became infective, and remained so for at least 49 days. On the 56th day the remaining 12 flies were dissected and examined for flagellates. None were found, but the contents of the alimentary canals of the 12 flies, pooled and injected into a healthy monkey, gave rise to Sleeping Sickness.

Experiment 1549.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Aug. 23—27	1—4	Flies fed on heavily-infected monkey			50 flies used.
„ 28—29	5—6	Flies starved.			
„ 30—Oct. 5	7—43	Flies fed on healthy monkey, 1617	+		Oct. 8, 20 remaining flies dissected, 4 found infected.
Oct. 6—7	44—45	Flies starved.			

Remarks.—Fifty laboratory-fed flies were fed on an infected monkey daily for five days. Thirty-seven days after their first feed they became infective. Twenty flies remained alive on the 46th day, and on dissection four were found infected with flagellates. A drop of fluid from the alimentary canals of two of these infected flies injected into a monkey gave rise to Sleeping Sickness.

Experiment 1566.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Aug. 29—Sept. 1	1—3	Flies fed daily on infected ox			35 flies used.
Sept. 2—3	4—5	Flies starved.			Oct. 21, 20 remaining flies killed and dissected; 9 found infected.
„ 4—Oct. 19	6—51	Flies fed on healthy monkey, 1566	+		
Oct. 20	52	Flies starved.			

Remarks.—Thirty-five laboratory-bred flies were fed daily for four days on an ox infected with *Trypanosoma gambiense.* Fifty-three days after their first feed the flies became infective. Nine of the 20 remaining flies, when dissected, showed infection with flagellates: three of these had infection of the proboscis. The contents of the alimentary canal of one infected fly injected into a monkey and goat gave negative results, as also did the probosces of two infected flies when injected into a goat.

Experiment 1602.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 1—4	1—3	Flies fed on infected ox daily			50 flies used.
„ 5—6	4—5	Flies starved.			
„ 7—14	6—13	Flies fed on healthy monkey, 1620		—	
„ 15—20	14—19	Flies fed alternately, daily, on healthy monkey, 1620, and goat, 1690		—	
„ 21—29	20—28	Flies fed alternately, daily, on healthy monkey, 1703, and goat, 1690	1703 +	1690 —	Sept. 29, 32 remaining flies killed by accident and dissected; 5 found infected.

Remarks.—Fifty laboratory-bred flies were fed daily for four days on an infected ox, as in the previous experiment. From the 15th to the 28th day the flies were fed alternately on a monkey and goat. Twenty-seven days after their first feed the flies infected monkey 1703 with Sleeping Sickness. Five of the 32 remaining flies showed infection with flagellates, and one of these injected into a monkey gave rise to Sleeping Sickness.

Experiment 1651.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 11—14	1—3	Flies fed on infected monkey			60 flies used.
„ 15—16	4—5	Flies starved.			Monkey 1651 died on 13th day.
„ 17—24	6—13	Flies fed on healthy monkey, 1651		—	Nov. 1, 32 remaining flies killed and dissected; 4 found infected.
„ 25—Oct. 29	14—48	Flies fed on healthy monkey, 1720	+		
Oct. 30—31	49—50	Flies starved.			

Remarks.—Sixty laboratory-bred flies were fed daily for four days on an infected monkey. Forty-one days after their first infected feed they became infective. Four flies infected with flagellates were found among the 32 which remained alive on the 51st day. They were not injected into animals.

Experiment 1712.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 23—Oct. 5	1—12	Flies fed on infected monkey			50 flies used.
Oct. 6—7	13—14	Flies starved.			Nov. 4, 31 remaining flies killed and dissected; 1 found infected.
„ 8—Nov. 2	15—40	Flies fed on healthy monkey, 1790	+		
Nov. 3	41	Flies starved.			

Remarks.—Fifty laboratory-bred flies were fed daily for 13 days on an infected monkey. The flies became infective 32 days after their first feed. On dissection of the remaining 31 flies on the 41st day, one was found to be infected with flagellates, but it did not give rise to the disease when injected into a healthy monkey.

Experiment 1760.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Oct. 1—5	1—4	Flies fed on infected monkey			60 flies used.
„ 6—7	5—6	Flies starved.			Nov. 4, 1 infected fly injected into healthy monkey.
„ 8—Nov. 6	7—36	Flies fed on healthy monkey, 1760	+		
Nov. 7	37	Flies starved.			Nov. 8, 28 remaining flies killed and dissected; all negative.

Remarks.—Sixty laboratory-bred flies were fed daily for five days on an infected monkey. They became infective on the 29th day. On the 34th day one fly which had died was found to be infected with flagellates, but on injection into a monkey it failed to give rise to Sleeping Sickness. No infected flies were found among the 28 remaining alive on the 38th day, when they were dissected.

Experiment 1761.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Oct. 1—5	1—4	Flies fed on infected monkey			50 flies used.
„ 6—7	5—6	Flies starved.			
„ 8—Nov. 8	7—38	Flies fed on healthy monkey, 1761	+		Nov. 11, 31 remaining flies killed and dissected; all negative.
Nov. 9—10	39—40	Flies starved.			

Remarks.—Fifty laboratory-bred flies were fed daily for five days on an infected monkey. They became infective on the 38th day. No infected flies were found among the 31 remaining alive on the 41st day, when they were dissected.

Table IV.—Number of Flies found Infected with *Trypanosoma gambiense* in the Experiments with Laboratory-Bred Flies.

Experiment.	No. of flies used.	Experiment, positive or negative.	No. of infected flies found.	Result of injection of infected flies.	Remarks.
975	16	+	0	Positive	12 remaining flies pooled.
1269	22	—	2	Negative.	
1452	90	—	1	„	
1549	50	+	4	Positive.	
1566	35	+	9	Negative	1 fly injected.
1602	50	+	5	Positive.	
1651	60	+	4		Flies not injected.
1672	28	—	2	Negative.	
1680	45	—	1	„	
1693	50	—	2	„	
1706	60	—	4	„	
1712	50	+	1	„	
1760	60	+	1	„	
1769	60	—	2	Positive.	
1801	70	—	1		Fly not injected.

There are some curious results to be noted here. In Experiment 975 the twelve remaining flies were dissected and examined. None was found infected. They were then pooled and injected into a healthy monkey, which became infected with Sleeping Sickness. This shows that the infected fly may escape detection by the microscope.

In Experiments 1269, 1452, 1672, 1680, 1693, and 1706, flies were found containing flagellates. These flies had not given rise to disease in the monkey they had been fed on, nor did their injection prove successful. The flagellates must be considered to be *Trypanosoma gambiense*, and therefore a cage of flies may become infected without causing disease either by biting or injection.

In Experiments 1549 and 1602, flies were found containing flagellates, and these flies succeeded in infecting monkeys both by biting while alive and injection after death.

Lastly, in Experiments 1566, 1712, and 1760, flies were found with flagellates which had infected the monkey fed on, but which failed to give rise to disease when their body-contents were injected into healthy animals.

In these experiments 746 laboratory-bred flies were used. Thirty-nine became infected—that is to say, more than 5 per cent.

C. *The Development of* Trypanosoma dimorphon *in Lake-shore* Glossina palpalis.

This is the commonest cattle trypanosome in Uganda. During 1909 it caused epidemics among the Government transport oxen at Entebbe, Mr. Walsh's cattle at Kabula Muliro, and the Uganda Company's cattle at Namukekera, all of which were investigated by the Commission.

The name *Trypanosoma dimorphon* is used for this species, although two forms have not been found. It belongs to the short, stumpy type of trypanosomes, without free flagella, and is probably identical with that found in Zanzibar by Edington; in Portuguese East Africa, described by Theiler; in Northern Rhodesia by Montgomery and Kinghorn; and in Southern Rhodesia by Bevan.

Table V.—Development of *Trypanosoma dimorphon* in Lake-shore *Glossina palpalis.*

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
574	500	3	21	21
996	100	3	—	—
1010	120	3	—	—
1022	100	3	—	—

Only one experiment out of four was successful. The flies became infective 21 days after their first feed on the infected dog.

Experiment 574.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Mar. 4—6	1—2	Flies fed on infected dog			500 flies used.
„ 7—25	3—21	Flies fed on a fowl.			
„ 26—30	22—26	Flies fed on healthy monkey, 649	+		Mar. 25, 120 flies alive,
„ 31—Apr. 3	27—30	Flies fed on healthy monkey, 650		—	Apr. 14, 60 flies alive.
Apr. 4—8	31—35	Flies fed on healthy monkey, 660		—	May 3, 30 flies alive.
„ 9—13	36—40	Flies fed on healthy monkey, 678		—	May 14, 22 remaining flies dissected; all negative.
„ 14—May 13	41—70	Flies fed on healthy monkey, 723		—	

Remarks.—Five hundred wild flies were fed for three days on a dog whose blood contained numerous *Trypanosoma dimorphon.* Twenty-one days after their first feed they became infective. By the 27th day they lost the infection and did not regain it, although kept under observation for 71 days. None of the flies which died or were killed and dissected showed any flagellates. It appears as if the infected fly had died early in the experiment and had escaped notice.

D. *The Development of* Trypanosoma dimorphon *in Laboratory-Bred* Glossina palpalis.

Table VI.—Development of *Trypanosoma dimorphon* in Laboratory-Bred *Glossina palpalis.*

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
1642	50	4	14	14
1675	50	3	—	—
1676	50	3	—	—
1843	140	4	—	—

Four experiments were carried out, as in the Lake-shore group, and one also was successful. The flies became infective on or about the 14th day.

Experiment 1642.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Sept. 8—11	1—3	Flies fed on infected oxen			50 flies used.
„ 12—13	4—5	Flies starved.			
„ 14—28	6—20	Flies fed on healthy ox, 870	+		
„ 29	21	Flies starved.			
„ 30—Oct. 11	22—33	Flies fed on healthy monkey, 1741		—	Oct. 13, 35 remaining flies dissected; all negative.
Oct. 12	34	Flies starved.			

Remarks.—Fifty laboratory-bred flies were fed on two infected oxen for four days, and then on a healthy ox. Fourteen days after their first infected feed this ox took the disease.

These experiments with *Trypanosoma dimorphon* are not very satisfactory. Experiment 574 appears to be fairly free from fallacy, and from it, it would seem probable that *Trypanosoma dimorphon* can develop in *Glossina palpalis* and infect a healthy animal after a period of 21 days. Ox 870, in Experiment 1642, became infected at a time when several of the cattle at Mpumu became naturally infected with this trypanosome disease, so that this experiment is not free from doubt. It is evident that more work must be done before anything definite can be said regarding this species.

E. *The Development of* Trypanosoma vivax *in Lake-shore* Glossina palpalis.

Table VII.—Development of *Trypanosoma vivax* in Lake-shore *Glossina palpalis.*

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
997	60	4	11	48
998	45	4	11	44
1014	200	4	21	60 ?

As *Trypanosoma vivax* does not affect monkeys, naturally cattle or goats were used in these experiments. The three experiments with Lake-shore flies were all successful; two became infected in 11 days, and one in 21 days.

Here follow the experiments in detail :—

Experiment 997.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
June 15—18	1—3	Flies fed on infected goat			60 flies used.
„ 19—20	4—5	Flies starved.			
„ 21—July 3	6—18	Flies fed on healthy calf, 1030	+		July 6, injected infected fly into goat; negative.
July 4—5	19—20	Flies starved.			
„ 6—21	21—36	Flies fed on healthy bull, 1268	+		Aug. 10, remaining flies dissected. Infected fly injected into goat; negative.
„ 22—23	37—38	Flies starved.			
„ 24—Aug. 10	39—56	Flies fed on healthy calf, 737	+		

Remarks.—Sixty wild flies were fed for four days on a goat infected with *Trypanosoma vivax*. Eleven days after their first feed they became infective, and remained so during the experiment. Two flies were found infected with flagellates, one on the 21st day, and one on the 56th day, both of which when injected into goats failed to give rise to the disease.

Experiment 998.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
June 15—18	1—3	Flies fed on infected goat			45 flies used.
„ 19—20	4—5	Flies starved.			Aug. 6, pooled contents of 14 flies remaining; injected into goat, which became infected with *T. vivax*.
„ 21—July 3	6—18	Flies fed on healthy calf, 1030	+		
July 4—5	19—20	Flies starved			
„ 6—Aug. 5	21—51	Flies fed on healthy calf, 1267	+		

Remarks.—Forty-five wild flies were fed for four days on an infected goat. Eleven days after their first feed they became infective, and remained so during the experiment. The pooled contents of the 14 remaining flies injected into a goat on the fifty-second day gave rise to infection with *Trypanosoma vivax*.

Experiment 1014.

Date.	Day of experiment.	Procedure.	Results.		Remarks.
			Positive.	Negative.	
1909. June 18—22	1—4	Flies fed on infected goat			200 flies used.
„ 23—24	5—6	Flies starved.			July 26, injected infected fly into a goat and monkey, the former of which became infected.
„ 25—July 17	7—29	Flies fed on healthy goat, 1079	+		
July 18	30	Flies starved.			
„ 19—Aug. 3	31—46	Flies fed on healthy goat, 1344	+		Aug. 4, 17 remaining flies dissected; all negative.

Remarks.—Two hundred wild flies were fed for five days on an infected goat. Twenty-one days after their first feed they became infective, and remained so during the experiment. On the 38th day one infected fly was found, which on injection into a goat and a monkey gave rise to *Trypanosoma vivax* infection in the former animal. Seventeen flies remained alive at the end of the experiment, and were killed and dissected. None of them was found to harbour flagellates.

Table VIII.—Number of Flies found infected with Trypanosomes in the Experiments with Lake-shore Flies and *Trypanosoma vivax.*

Experiment.	No. of flies used.	Experiment, positive or negative.	No. of infected flies found.	Result of injection of infected flies.	Remarks.
997	60	+	2	—	
998	45	+	2	+	14 flies remaining pooled.
1014	200	+	1	+	

Of the above three positive experiments it is seen that five infected flies were found. One of these—Experiment 1014—when injected into a susceptible animal gave rise to *Trypanosoma vivax* infection. In Experiment 998, among the 14 remaining flies, two were found with trypanosomes in their probosces. None of the 14 showed flagellates in the gut. The body-contents of the 14 flies, in addition to the contents of the two probosces, were pooled and injected into a goat, which 12 days afterwards showed *Trypanosoma vivax* in its blood.

F. *The Development of* Trypanosoma vivax *in Laboratory-Bred* Glossina palpalis.

Table IX.—Development of *Trypanosoma vivax* in Laboratory-Bred *Glossina palpalis*.

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
1591	50	4	21	21
1638	25	6	—	—
1698	68	4	35	35
1700	60	4	30	30
1870	50	?	—	—

Five experiments were made with laboratory-bred flies. Three were successful.

Experiment 1591.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 2—4	1—2	Flies fed on infected calf, 1318			50 flies used.
„ 5—6	3—4	Flies starved.			
„ 7	5	Flies fed on infected calf, 1318.			Oct. 4, 35 remaining flies dissected; negative except 3, which had infected probosces.
„ 8—9	6—7	Flies starved.			
„ 10—30	8—28	Flies fed on healthy goat, 1652	+		
Oct. 1—3	29—31	Flies starved.			

Remarks.—Fifty laboratory-bred flies were fed for four days on an infected calf; 21 days after their first feed they became infective; 35 flies remained alive on the 32nd day. On these being dissected three were found with infected probosces. These probosces were not injected into animals, so that it is not known if the flagellates were infective by injection.

Experiment 1638.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909.					
Sept. 6—11	1—5	Flies fed on infected calf			25 flies used. Sept. 29, 23 remaining flies accidentally killed; negative on dissection.
„ 12—13	6—7	Flies starved.			
„ 14—29	8—23	Flies fed on healthy goat, 1682		—	

Remarks.—Twenty-five laboratory-bred flies were fed for six days on a calf infected with *Trypanosoma vivax*, and afterwards for 16 days on a healthy goat. This goat was not infected, and the remaining 23 flies when killed and dissected all proved negative.

Experiment 1698.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Sept. 21—24	1—3	Flies fed on infected ox			68 flies used. Calf 1893 died 29.11.09.
„ 25—26	4—5	Flies starved.			
„ 27—Nov. 5	6—45	Flies fed on healthy ox, 425	+		Nov. 12, 53 flies remaining alive dissected; probosces of 5 swarming with flagellates.
Nov. 6—9	46—49	Flies fed on healthy calf, 1893		—	
„ 10—11	50—51	Flies starved.			

Remarks.—Sixty-eight laboratory-bred flies were fed for four days on an ox whose blood contained *Trypanosoma vivax*. About the 35th day the flies became infective. On the 52nd day the 53 flies which remained alive were killed and dissected. The probosces of five, three males and two females, were found to be swarming with flagellates. These were not injected into animals.

Experiment 1700.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. Sept. 21—24	1—3	Flies fed on infected ox			60 flies used.
„ 25—26	4—5	Flies starved.			
„ 27—Oct. 28	6—37	Flies fed on healthy calf, 290	+		Oct. 30, 38 remaining flies dissected; 22 found infected.

Remarks.—Sixty laboratory-bred flies were fed for four days on an ox whose blood contained *Trypanosoma vivax*. About the 30th day these flies became infective. On the 39th day the 38 remaining flies were killed and dissected. There were 19 males and 19 females; 22 showed infection of the proboscis with crithidia-like flagellates. Only one fly showed flagellates in the gut. Of the 22 flies which had trypanosomes in the proboscis, 9 were males and 13 were females; 10 of the infected probosces were ground up in salt solution and injected into an ox; 12 days afterwards trypanosomes appeared in the blood of this ox.

Experiment 1870.

Remarks.—Fifty laboratory-bred flies were fed on a calf whose blood contained *Trypanosoma vivax*; 10 and 12 days afterwards all the flies were killed and dissected. No flagellates were found in any part of the flies.

Table X.—Number of Flies found Infected with Trypanosomes in the Experiments with Laboratory-Bred Flies and *Trypanosoma vivax.*

Experiment.	No. of flies used.	Experiment, positive or negative.	No. of infected flies found.	Result of injection of infected flies.	Remarks.
1591	50	+	3	Not injected	Probosces infected.
1698	68	+	5	,,	,, ,,
1700	60	+	22	+	,, ,,

In these experiments 178 flies were used, and of these 30, or 17 per cent., became infected. A curious fact is that in all the 30 flies, with the exception of one, the infection was confined to the proboscis. There was a feeling in the minds of the members of the Commission that this growth of flagellates in the proboscis was something quite characteristic of *Trypanosoma vivax.* Only on one occasion was this development of trypanosomes in the proboscis seen after feeding laboratory-bred flies on blood which was known to contain nothing but *Trypanosoma gambiense.*

G. *The Development of* Trypanosoma nanum *in Lake-shore* Glossina palpalis.

Table XI.—The Development of *Trypanosoma nanum* in Lake-shore *Glossina palpalis.*

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
1035	120	3	3	3

Only one experiment was carried out with *Trypanosoma nanum* and Lake-shore *Glossina palpalis.* It is unsatisfactory, as trypanosomes appeared in the first healthy goat a few days after the fly had fed on the infected animal. None of the flies which were dissected showed any flagellates in their alimentary canal.

Experiment 1035.

Date.	Day of experiment.	Procedure.	Result.		Remarks.
			Positive.	Negative.	
1909. June 21—23	1—2	Flies fed on infected goat.			120 flies used.
„ 24—25	3—4	Flies starved.			
„ 26—July 1	5—10	Flies fed on healthy goat, 1171	+		
July 2—4	11—13	Flies starved.			Aug. 3, goat 1257 died; negative.
„ 5—Aug. 2	14—42	Flies fed on healthy goats, 1257 and 1258.			Aug. 25, 9 remaining flies dissected; negative.
Aug. 3—24	43—64	Flies fed on goat 1258		—	

Remarks.—One hundred and twenty wild flies were fed for three days on an infected goat. Five days after their first feed they infected a healthy goat, or, at least, trypanosomes resembling *Trypanosoma nanum* appeared in the blood. They failed to infect other healthy goats, although they were fed up to the 64th day after their first infected feed. Nine remaining flies dissected on the 65th day were negative for flagellates.

H. *The Development of* Trypanosoma nanum *in Laboratory-Bred* Glossina palpalis.

Table XII.—The Development of *Trypanosoma nanum* in Laboratory-Bred *Glossina palpalis.*

Experiment.	No. of flies.	No. of days fed on.	No. of days before flies became infective.	No. of days flies remained infective.
1738	100	3	—	—

Only one experiment was carried out with laboratory-bred flies. The result was negative.

Experiment 1738.

Remarks.—One hundred laboratory-bred flies were fed for three days on an ox whose blood contained *Trypanosoma nanum*, and then on a healthy goat for 40 days. This goat remained healthy, and all the flies when dissected were found free from flagellates.

A few experiments were made by the Commission on the development of *Trypanosoma brucei* and *Trypanosoma cazalboui* in the Sleeping Sickness tsetse fly, *Glossina palpalis*, but they came to nothing.

Conclusions.

1. That *Trypanosoma gambiense* multiplies in the gut of about one in every 20 *Glossina palpalis* which have fed on an infected animal.

2. That the flies become infective, on an average, 34 days after their first feed.

3. That a fly may remain infective for 75 days.

4. That *Trypanosoma dimorphon*, *Trypanosoma vivax*, and *Trypanosoma nanum* may also multiply in *Glossina palpalis*, which must therefore be looked upon as a possible carrier in these diseases.

5. That multiplication in the tube of the proboscis is characteristic of *Trypanosoma vivax*.

Colour Blindness and the Trichromatic Theory of Colour-vision.

By Sir WILLIAM DE W. ABNEY, K.C.B., D.Sc., D.C.L., F.R.S.

(Received January 21,—Read February 24, 1910.)

[This paper is published in Series A (No. 565), vol. 83.]

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THE SOLUBILITY OF XENON, KRYPTON, ARGON, NEON, AND HELIUM IN WATER. By A. VON ANTROPOFF, University College, London, 1908; Polytechnikum, Riga, 1909 .. 471

THE INFLUENCE OF PRESSURE ON THE BOILING POINTS OF METALS. By H. C. GREENWOOD, M.Sc., Beyer Fellow of the University of Manchester 483

THE SCATTERING OF THE α-PARTICLES BY MATTER. By H. GEIGER, Ph.D. 492

THE IONISATION PRODUCED BY AN α-PARTICLE. PART II.—CONNECTION BETWEEN IONISATION AND ABSORPTION. By H. GEIGER, Ph.D. 505

ON THE VISCOSITIES OF THE GASES OF THE ARGON GROUP. By A. O. RANKINE, B.Sc., Assistant in the Department of Physics, University College, London 516

THE CHANGE OF CARBON DISULPHIDE INTO A GASEOUS PRODUCT CONDENSABLE AND EXPLOSIVE NEAR THE TEMPERATURE OF LIQUID AIR. By Sir JAMES DEWAR, M.A., Sc.D., LL.D., F.R.S., Jacksonian Professor in the University of Cambridge; and HUMPHREY OWEN JONES, M.A., D.Sc., Jacksonian Demonstrator in the University of Cambridge 526

THE IONISATION OF VARIOUS GASES BY THE β-RAYS OF ACTINIUM. By R. D. KLEEMAN, D.Sc., B.A., Mackinnon Student of the Royal Society 530

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4888

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BIOLOGICAL SCIENCES.

CONTENTS.

	Page
The Thyroid and Parathyroid Glands throughout Vertebrates. By F. D. THOMPSON. (Abstract)	389
Tone Perception in Gammarus pulex. By F. J. COLE, B.Sc., Professor of Zoology, University College, Reading	391
A Physiological Effect of an Alternating Magnetic Field. By SILVANUS P. THOMPSON, D.Sc., F.R.S.	396
On the Weight of Precipitate Obtainable in Precipitin Interactions. By Dr. H. G. CHAPMAN	398
The Influence of Bacterial Endotoxins on Phagocytosis. (Preliminary Report.) By LEONARD S. DUDGEON, P. N. PANTON, and H. A. F. WILSON	406
A Case of Sleeping Sickness Studied by Precise Enumerative Methods: Regular Periodical Increase of the Parasites Disclosed. By Major RONALD ROSS, F.R.S., and DAVID THOMSON, M.B., Ch.B., D.P.H.	411
On the Recognition of the Individual by Hæmolytic Methods. (Preliminary Communication.) By CHARLES TODD, M.D., Bacteriologist, Egyptian Government, and R. G. WHITE, M.B., Director, Serum Institute, Cairo	416
Experimental Researches on Vegetable Assimilation and Respiration. VI.—Some Experiments on Assimilation in the Open Air. By D. THODAY, M.A., Junior University Demonstrator in Botany, Cambridge	421
Receptors and Afferents of the Third, Fourth, and Sixth Cranial Nerves. By FRANCES M. TOZER, B.Sc., and C. S. SHERRINGTON, D.Sc.	450
The Relation of Light Perception to Colour Perception. By F. W. EDRIDGE-GREEN, M.D., F.R.C.S., Beit Medical Research Fellow	458

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The Thyroid and Parathyroid Glands throughout Vertebrates.

By F. D. Thompson.

(Communicated by Prof. E. A. Schäfer, F.R.S. Received December 29, 1909,—
Read February 3, 1910.)

(Abstract.)

1. The organs recognised as arising in the regions of the gill-clefts in Elasmobranchs are thyroid, thymus, and post-branchial body. The parathyroid and carotid glandules have not yet been discovered in these animals.

2. Within the thyroid gland of Elasmobranchs are small, solid masses of cells, partly epithelial, partly adenoid. These have not, so far as I am aware, been previously described. One is tempted to suppose that these are homologous either with parathyroid or thymus. In the latter case it would correspond with thymus IV of Mammals. (It has not been suggested that the thymus derivative of the fourth cleft furnishes isolated nodules in the thyroid of Elasmobranchs.)

3. In Teleosts the only organs of the series are the thyroid and the thymus. The parathyroid has never been described in this group, and it is doubtful whether there is any trace of post-branchial body. The thyroid in *Amiurus* consists of a few scattered vesicles embedded in the connective tissue matrix. The cells lining the vesicles are very low columnar, and in some cases almost flat.

4. In Urodela the branchial cleft organs are thyroid, thymus, parathyroid, and post-branchial body. The thyroid is fairly superficial, and there is no intimate relation with the parathyroid.

5. In Anura the branchial organs are, in addition to thyroid and parathyroid, thymus, post-branchial body, ventral "Kiemenreste" and carotid gland. The thyroid is very small and deeply placed. The parathyroid has not yet entered into intimate relations with the thyroid. The ventral "Kiemenrest" is a large striking-looking organ which must have been frequently mistaken for the thyroid, and appears to be a hæmolymph organ. The arrangement of the cells in the parathyroid is somewhat characteristic, and is described in the text.

6. In Reptiles, thyroids and parathyroids are still anatomically separate organs, but the parathyroid in some instances possesses distinct lumina, and in this the fundamental distinction between thyroid and parathyroid is at once broken down. Moreover, in this group there are clear indications that the post-branchial body secretes colloid.

7. In Birds, we frequently find large areas of the thyroid either devoid of colloid vesicles, or having these in a compressed, crowded condition. But the parathyroids are still separate and distinct organs. The post-branchial body presents certain peculiar features, described in the text, among these being an accumulation of concentric corpuscles, such as is found in the epithelial part of the thymus.

8. In Mammals, there is much more intimate relationship between the parts of the thyroid apparatus than in lower animals. The cells lining the vesicles are practically of the same character as those accumulated in varying amount between the vesicles, and do not differ in any essential respect from those forming the parathyroid glandules.

Many of the masses of intervesicular cells are indistinguishable from parathyroids. The internal parathyroid is frequently in direct tissue continuity with the thyroid, and every kind of transition form exists. Parathyroid has only, indeed, to have colloid spaces in its interior to constitute itself thyroid, and this occurs in the human subject under certain pathological conditions.

Parathyroids left behind after removal of the thyroid develop colloid vesicles and become practically converted into thyroid. Moreover, the changes in the thyroid, after removal of the parathyroids, may be interpreted as the reverse change of thyroid into parathyroid tissue. The experimental evidence as to a separate function for parathyroids is inconclusive.

9. Thyroid and parathyroids are to be looked upon as structures of somewhat different embryological origin, which are anatomically separate and distinct in the lower Vertebrata, but which come into very intimate anatomical and physiological relationships with each other in the Mammalia. In this latter group they are, in fact, to be looked upon as constituting one apparatus.

10. Parathyroidectomy, like thyroidectomy, causes compensatory changes in the pituitary body. These consist in a notable increase of the colloid vesicles in the *pars intermedia.*

This observation confirms the general view that thyroid and parathyroid are very intimately related to each other. The colloid vesicles of the *pars intermedia* of the pituitary resemble in all respects those of the thyroid and of the parathyroid (where such occur), and it is probable that the intermediate portion of the pituitary is to be looked upon as an integral part of the thyroid —parathyroid apparatus.

Tone Perception in Gammarus pulex.

By F. J. Cole, B.Sc., Professor of Zoology, University College, Reading.

(Communicated by A. E. Shipley, M.A., F.R.S. Received January 17,—
Read April 21, 1910.)

The proof of the existence of a restricted tonal sense in any animal would be of great interest. The present paper offers evidence of the occurrence of this phenomenon in the Gammarids. Though it must be admitted that the number of animals which responded to the tests was small, the response, when it occurred, was so definite and unmistakable that a description of the results seems desirable. My attention was first directed to the matter by Miss Margaret Cussans, who was engaged at the time in working out, at my suggestion, the circulation of *Gammarus pulex* on the living animal under the microscope. We then noticed that this species responded only to the bass note of the College chime, which, giving on examination 240 vibrations per second, therefore corresponded to the B below middle C of the pianoforte.

In the first place it must be noted that the animal responds (in water) only when imprisoned in the compressorium or live box. This makes the case the more interesting, since if the reaction had not been observed by the merest accident, the same experiments might have been conducted on the animals in their natural surroundings with absolutely negative results. They are compressed only so much as to prevent wandering, whilst leaving their appendages quite free. They may then be kept under observation on the stage of the microscope. I had a special live box constructed so as to permit a gentle stream of water being passed continuously through it. This prevents asphyxiation, and allows a single specimen to be experimented with for a longer period than would otherwise be possible. It is, of course, inevitable that the vibrations should be conveyed to the animal by the medium in which it finds itself, and in this sense it is impossible to postulate true audition (as usually understood) in any true aquatic species. The factor to eliminate here is clearly the specific period of the medium itself, the energetic vibration of which, in response to its own note, may vitiate the result of the experiment. For this reason I used three types of live box and microscope, without, however, any difference in the results being noticed. Further, the fact that the response is only evoked by a limited range of tone, and then without reference to any specific forced or sympathetic vibration of the surrounding medium, intimates that the stimulus is associated with a definite physiological status of the animal itself, since even if the box were an essential factor in the success of the test, we should still leave unexplained the differential response. It is, however, easy to show that the box itself, owing to its small size and heavy construction, is ill adapted for the transmission of vibrations, and is certainly the last means one would adopt to transmit aerial vibrations freely to the animal.

Tested with the trombone under the conditions of many of the following experiments, both by means of the optical lever and by polarised light, two of the live boxes showed no traces of strain in the latter case, and in the former only an extremely slight vibration to the G, F sharp, and F below middle C.

The animal having been placed in the live box, and the current of water started, the whole was then transferred to the stage of the microscope, and the following three tests applied:—

(1) On a specially prepared physical table, constructed of alternate layers of sawdust and three massive cast iron slabs,* which served to filter off vibrations from the ground, and to ensure that any response observed was due to a stimulus conveyed through the atmosphere, *i.e.* was a true auditory and not a tactual reaction. Judged by the mercury test this table was quite efficient.

(2) On an elongated rectangular resonator provided with a sliding piston. Here the rationale of the piston is to make it possible to eliminate the factor of the specific note of the resonator itself (a point of obvious importance), and I may as well state at once that the position of the piston never appeared to affect the results in the least. For example, a female tested on the resonator responded equally well to middle B flat whilst the length of the resonator was being reduced from 135 to 30 cm. I should also observe here that the bell of the trombone was usually placed near the open end of the resonator, but not, of course, touching it.

(3) On an organ reed, made to my instructions, which gave at least two good octaves, one above and the other below middle C. This was effected by means of a sliding piston and an adjustable lip, and was much more satisfactory than the makers anticipated that it would be.

In the cases of 1 and 2, a B flat or tenor trombone was employed to provide the stimulus, and it was generally found necessary to produce a *ff* note. I have observed, for example, that a crescendo note is only responded to when the louder portion is reached.

The intervals mentioned in this paper are those of the harmonic chromatic scale of C major.

The responses which the animal makes to sound are of two kinds, and although there can be no doubt as to the genuine character even of the first, it is only to the second that I have considered it safe to attach any importance. In the one case the first antennæ are observed to give a distinct neurotic flicker, but in the other they are rapidly and powerfully flexed, being bent right underneath the body, and carrying the passive second pair of antennæ before them. In both cases it is important to note that we have to do with a definite *physiological* response, which unquestionably supervenes as the result of the stimulus, and is not a merely physical effect such as Hensen observed in the auditory setæ of the Decapod Crustacea, which effect has since been shown to have no *necessary* auditory significance. In my earlier experiments no record was, unfortunately, made of the sex of the specimens employed. This was, however, done in the later work, but no difference between the sexes was observed. In a very few cases the response was of a character different from either of those just described, the anterior antennæ being only partially flexed at the first stimulus, and, instead of returning to the *status quo,* becoming more and more flexed with each successive stimulus, until they finally reached a position similar to that attained by the one flexure in the case of the second type of response above. Tested dry out of water directly on the resonator, and on the organ pipe, some individuals showed very distinct signs of disturbance to notes immediately above and below middle C, but they did not hop.

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* My colleague, Prof. G. J. Burch, F.R.S., kindly suggested this to me.

In *Gammarus* the auditory function is generally supposed to be located in the first antennæ, and, that this is so, is proved by the following experiments. Four pairs *in copula* were separated, and two of each sex had either the first or the second pair of antennæ removed. This is easily done by holding the animal down with a camel's hair brush and detaching the antennæ with a sharp needle or a very fine pair of forceps. The blood at once clots over the wound, leaving a characteristic black scar. Both males and females on the resonator responded to middle B flat after removal of the second antennæ, in some cases only to the B flat, and in one case markedly to the B flat, and less so to a few semitones below it. On no occasion, however, was the slightest response observed after the first antennæ had been removed. A female minus the second antennæ was tested on the physical table, and responded distinctly to B flat when the bell of the trombone was not more than 6 inches from the microscope, but failed to respond when the bell was withdrawn to 18 inches. All the above individuals were tested again 10 days after the operation (except one female which had died), and whilst there was still no response whatever from those without the first antennæ, those with these appendages only responded in two cases, and then but slightly.

Fatigue appears to play an important part in the results of the experiments, and this I have noticed a number of times. For example, a specimen in the live box placed on the resonator responded very markedly to B flat five successive times. Transferred then to the physical table it responded energetically to B flat the first time, not so distinctly the second time, slightly the third time, very distinctly the fourth time, and slightly the fifth time. Replaced now on the resonator, it no longer responded either to B flat or A flat. As it seemed possible that the shock of compressing the animal in the live box and testing it at once might affect the results, I tried the effect of several hours' imprisonment in the live box before applying the stimulus. I could not, however, persuade myself that there was any appreciable difference. For example, a female was placed in the live box, and the current of water started at 11 A.M. The heart beat was then 210 per minute.* Tested on the resonator first at 135 cm. and then at 85 cm. at 2.30 P.M., when the heart beat was 240 per minute, it responded quite well at first, but then failed to respond on the physical table, and afterwards on the resonator.

A typical, successful experiment may be described as follows: A male was tested on the resonator (but without the water current) with the chromatic scale up and down from middle B flat. Between every two notes the B flat was sounded in order to make it certain that a negative result was not due to fatigue or other causes. The response to B flat was very energetic, the first antennæ making a sudden and rapid downward sweep under the body, carrying the second antennæ with them. There were also responses, but not so well marked, down to the A flat, and slight doubtful responses three semitones below that again, but no more. In all cases, however, there was a quite perceptible difference between the response to the alternative B flats and the other notes of the scale. On the animal showing signs of fatigue the alternative B flat chromatic scale was repeated, but now there was only a response to the B flat. Tested to an octave above the middle B flat, there was only a slight response to the upper three semitones, but in no respect was it at all striking. Transferred now to the physical table the animal responded simply to the B flat, and then only when the bell of the trombone was quite near the microscope. This, however, must have been due to fatigue, as no better result was elicited when the experiment was subsequently tried on the resonator. In another case a male was tested on the resonator and with running water with the alternative B flat chromatic scale, a quarter of a minute rest being allowed between successive notes. The response to B flat was quite undoubted, to A natural very slight, and to A flat scarcely perceptible, but there was no further response down to F, at which time, the animal failing to respond even to the B flat, the experiment was brought to a conclusion.

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* Miss Cussans states that the heart beat ranges from 120 to 130 per minute.

A large number of specimens were tested with a view to ascertaining whether the sensitiveness to middle B flat was of general occurrence. For example, a male placed on the resonator responded moderately to A flat at the first stimulus, but very slightly if at all to the same note the next four times. Tried then with B flat and A flat sounded alternately, five times each, there was always a response to the B flat, quite distinct if not very well marked, but none at all to the A flat. The subordinate notes may be eliminated by gradually withdrawing the trombone to a distance. Thus a male on the resonator responded quite well when the bell of the instrument was 4, 7, and 10 inches from the mouth of the resonator, but did not respond at all after the foot had been passed. In another case there was a marked response when the bell was a foot away, and even slight responses at 1½ and 2 feet, but none beyond the latter distance. In a female, with the bell at a foot, there was a marked response to B flat, and only a slight one to A natural. Again, a female tested on the physical table responded distinctly to B flat at 4 inches, slightly at 8 inches, but distinctly again at 12 inches, and at 2 feet not at all. Returning now to 4 inches there was a well-marked response, and immediately at 2 feet a slight response.

A specimen in a remarkable condition of hyperæsthesia was encountered on one occasion. In the first experiment the chromatic scale was played with one minute rest between successive notes, but it should be pointed out that at first the animal also responded to the slightest tap on the resonator, the only individual which has done this. It was a male, and we tried it first on the resonator at 135 cm., and with the current of water. The result was as follows: To middle B flat and A natural there were at once very striking and emphatic responses; to A flat and G, rather less; to F sharp, F, E natural, and E flat, well-marked responses; to D natural, D flat, and C, not so well marked. We now repeated the same scale, but only allowed sufficient time between each note for the antennæ to straighten out. It responded in the same way to every note as before, but not so vigorously. Tested with the chromatic scale up from middle B flat, and with the same minute intervals, we found: B flat and B natural, well marked; C, less; D flat, very slight, *but responded well to the middle B flat immediately;* D natural and E flat, slight, but only slight to the B flat also; E natural, slight, but a better response to the B flat; F, very slight, but a much better response to the B flat. In the latter experiment the animal was, I think, probably becoming fatigued, and thus did not respond as well as it might have done at first. We now stopped the water current for a time, in the expectation of getting at the fundamental note of the animal by eliminating the subordinate tones. It still responded, but very slightly, to all the notes as before, but was unquestionably more sensitive about B flat and A flat.

I am, of course, aware that most of the preceding experiments may be interpreted as either tactual or auditory reactions, and, if it is possible at all to distinguish these two phenomena in the lower aquatic animals, the fact that they are not *necessarily* auditory does not justify one in concluding, as some recent observers have done, that they are therefore tactual. In the case, however, of the experiments conducted on the physical table, it is, to my mind, difficult to deny a true auditory sense to *Gammarus*. But this sense (whatever its nature) seems to be extremely restricted, and only a few tones appear to be appreciated.

A Physiological Effect of an Alternating Magnetic Field.

By Silvanus P. Thompson, D.Sc., F.R.S.

(Received April 4,—Read April 14, 1910.)

Down to the present time it has been held by all physicists and by all physiologists that magnetism produces no physiological effect either on the human subject or on any living organism. Many persons have looked for such effects. Lord Lindsay (now the Earl of Crawford), assisted by Mr. Cromwell F. Varley, constructed many years ago an enormous electro-magnet,* now in the Observatory at Edinburgh, so large that it would admit between its poles the head of any person who wished to test whether a strong magnetic field would have any sensible effect. Nothing whatever was perceived as the result.

I have, however, recently succeeded in demonstrating a real physiological effect due to magnetism. Some six years ago, when experimenting with an alternating electro-magnet which had been constructed for showing Prof. Elihu Thomson's well-known experiments on the repulsion of copper rings, I observed a faint visual effect when my forehead was placed close to the magnet.

Recently, incited thereto by finding Lord Kelvin's mention of the negative results in Lord Crawford's experiments, I have further explored the matter, and have found a means of producing the visual effect (which is physiological and subjective) in a way that succeeds with every person on whom it has yet been tried.

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* See Lord Kelvin's 'Popular Lectures and Addresses,' vol. 1, p. 261.

An alternating magnetic field of sufficient intensity and extent was produced by passing an alternating electric current around a specially constructed magnetising coil. This coil, consisting of 32 turns of stranded copper wire, having an equivalent cross-section of about 0·2 square inch in each turn, was wound upon a wooden cylinder about 9 inches in diameter, and about 8 inches in length; the cylinder being then removed. The alternating current, of a frequency of 50 periods per second, could be increased up to 180 amperes at will; so that the total number of ampere-turns reached 5760. The intensity of the alternating magnetic field at the centre of this coil had, therefore, a virtual value (quadratic mean) of about 1000 C.G.S. units; and its instantaneous maximum value (at the centre of the coil) was therefore about 1400 C.G.S. units. The value at the mouth of the coil was not much more than two-thirds of this amount, and was not uniform over the cross-section of the interior space.

On inserting the head into the interior of the coil, in the dark, or with the eyes closed, there is perceived over the whole region of vision a faint flickering illumination, colourless, or of a slightly bluish tint. The period of the flicker is not well defined. It does not seem to be the same over the whole region of vision at the same time, nor is it equally bright over the whole region of vision, but is somewhat brighter in the peripheral region than in the central parts. Even in daylight, with the eyes open, one is conscious of a sensation of flicker superposed upon the ordinary vision.

The effect is diminished by lowering the intensity of the field, and increased by raising it. Attempts to discover whether the brightness of the phenomenon stands in any relation to the direction of the axis of the field with respect to the principal axes of the skull have not yet revealed any definite result. It will be necessary to apply more intense fields than have yet been tried. No after-effects of any kind have been experienced either by myself or by any of the persons who have made the experiments with me.

As yet it has not been possible to ascertain whether varying the frequency has any effect on the phenomenon.

The phenomenon is so distinct, when once it has been seen, that it is difficult to believe that it has not been observed before by those who have been working with transformers. Nearly four years ago Prof. Birkeland, of Christiania, told me that his workmen at the nitrate factory at Notodden declared they could see lights over the choking coils used to limit the currents supplied to the electric furnaces. This may have been in reality a subjective phenomenon similar to that now recorded.

No effect upon the senses of smell, taste, or hearing has yet been observed.

[*Added April* 14.—Since the above was written, it has been noted by several observers that a sensation of taste in the mouth is excited after exposing the head for two or three minutes to the action of the alternating magnetic field.]

On the Weight of Precipitate Obtainable in Precipitin Interactions.

By Dr. H. G. Chapman.

(Communicated by Dr. C. J. Martin, F.R.S. Received April 21,—Read May 5, 1910.)

(From the Physiological Laboratory of the University of Sydney.)

The nature of the interaction between the antiserum and homologous protein in a precipitin test has been the subject of several researches. It has been usual to mix a given fixed quantity of antiserum with increasing quantities of suitably diluted homologous protein estimated either directly or in terms of dilutions and to measure the volume of the precipitate formed. The experiments of Hamburger which have been analysed by Arrhenius* were conducted in this way. Welsh and Chapman† also examined mixtures of a fixed quantity of antiserum with increasing quantities of homologous protein by adding to the superfluid above the precipitate either antiserum or homologous protein. This mode of testing the superfluid after the completion of the precipitin reaction led to certain definite conclusions. In the first place the further addition of antiserum to the superfluid always led to the formation of a further precipitate. In the second place it was possible to neutralise completely the precipitin in an antiserum, so that the further addition of homologous protein led to no more formation of precipitate.

The precipitin reaction between antigen and anti-body is very suitable for quantitative study owing to the simple nature of the interaction and the ease with which the quantities may be measured. More accurate methods are, however, required for its study than those previously employed. The present paper records the results of a gravimetric study of the reaction. The weights of the two interacting bodies and the weight of the final precipitate have been ascertained. The precipitin (anti-body present in the serum of the immunised animal) cannot be directly determined by weighing, as it forms only a small part of the dried antiserum. It will be assumed in the paper to be proportional to the volume of the antiserum. The homologous protein (either serum or egg-white) has been reckoned as milligrammes of dried serum or dried egg-white. The precipitate formed in the interaction has been weighed.

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* S. Arrhenius, 'Immuno-chemistry,' New York, 1907, p. 287 *et seq.*, and 'Ergebnisse d. Physiol.,' vol. 7, p. 543, 1908.

† 'Journ. of Hygiene,' vol. 6, p. 251, 1906.

The most striking feature of the results obtained is the strict proportion of the weight of the precipitate to the amount of the antiserum, provided the quantity of the homologous protein exceed certain minimal amounts. If a quantity of dried horse serum such as 50 mg. be allowed to interact in suitable dilution with 1, 2, 3 and 4 c.c. antiserum for horse serum the weights of the precipitates will be in the ratio of 1, 2, 3 and 4. This fact stands in harmony with the evidence obtained by Welsh and Chapman* concerning the origin of the precipitate mainly from the antiserum. The quantity of precipitate represents the "precipitable content" of the antiserum and its weight is practically that of the precipitin present in the antiserum.

Methods.

Rabbits and cats were employed to produce the antisera. They received six to eight injections of serum or egg-white into the abdominal cavity. The quantity of material injected was determined by drying quantities of serum or egg-white to constant weight. Approximately 1 gramme was given at at each injection. The animals were killed by bleeding 12 to 16 days after the final injection. The serum was allowed to separate spontaneously. This serum was utilised at once for the experiments. The whole serum from any immunised animal was mixed together before use, as it was found that the weight of the precipitate from the antiserum first separated differed considerably from that of the antiserum separated later. All vessels, glass tubes, measures and pipettes were sterilised by steam immediately before use. The saline solution (0·75 per cent. NaCl) was sterilised by boiling thrice on successive days. The antiserum was measured by a pipette carefully graduated by weighing the amount of mercury delivered. The sera and egg-whites used as homologous proteins were similarly measured after suitable dilution. The solid content of the serum or egg-white was estimated by drying to constant weight a measured volume. In this way it was more easy to ensure the absence of bacterial contamination. The quantities of the reacting bodies were mixed with saline solution in large tubes for the centrifuge. The tubes were made up to a fixed volume with saline solution and allowed to stand 48 hours for the interaction to take place. The superfluid above the precipitate was removed with a pipette and the precipitate washed five times with saline solution. Each time the precipitate was mixed with 50 c.c. saline solution and the precipitate separated by spinning in the centrifuge. The precipitate was then washed five times in the same way with 50 c.c. distilled water. The precipitate was transferred to small glass tubes with thin walls, weighing about 4 grammes. These tubes could be spun in a small centrifuge and, in this way, the precipitate was washed with absolute alcohol and finally with ether free from water. The tubes with their contents were placed in an oven at 80° C. for several hours and thence were put in a desiccator. The tubes were kept a fixed time in the desiccator and weighed. The tubes of a series were weighed immediately after each other. Owing to the hygroscopic nature of the precipitates the error in the weights of the tubes was found by experiment to be 0·3 milligramme. The mean figure of the several weighings was taken in all cases.

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* 'Roy. Soc. Proc.,' B, vol. 78, p. 297, 1906.

Experimental Results.

Experiments were performed to ascertain the weight of precipitate obtained when a measured quantity of antiserum was allowed to interact with increasing weights of homologous protein. It has been shown by Welsh and Chapman* that no precipitin can be detected in the superfluid at the end of an interaction, provided that the amount of homologous protein exceeds a certain quantity. In the series to be described the quantity of protein was sufficient to neutralise or precipitate† the precipitin in the antiserum. The superfluids were considered free from precipitin, since they yielded no precipitate on the addition of 144 milligrammes dried egg-white. The results are recorded in Table I.

Table I.

No. of tube.	Weight of dried egg-white.	Volume of antiserum.	Volume of saline solution.	Weight of precipitate.	Weight of precipitate from 1 c.c. antiserum.
	milligrammes.	c.c.	c.c.	milligrammes.	milligrammes.
1	14·4	2	50	3·2	1·6
2	36·0	2	50	3·5	1·75
3	144·0	2	50	3·4	1·7
4	432·0	2	50	3·4	1·7

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* 'Journ. of Hygiene,' vol. 6, p. 251, 1906.

† Compare Welsh and Chapman, 'Roy. Soc. Proc.,' B, vol. 78, p. 297, 1906.

Here the antiserum was formed by the injection of fowl's egg-white and the fresh antiserum allowed to interact with fresh egg-white. A portion of the egg-white was dried to determine the solid content of the solution. The precipitates are stated in terms of the amount yielded with 1 c.c. antiserum. The weight of precipitate remains practically constant, although the protein increases from 14 to 432 grammes. A somewhat similar series is recorded in Table II. In this series, 3 c.c. antiserum were allowed to interact with 70, 140, 280, and 560 milligrammes dried egg-white. After 24 hours the superfluids were removed, and to the superfluid of the tube No. 1 (1B in table), 70 milligrammes dried egg-white were added; to that of the tube No. 2 (2B in table), 140 milligrammes dried egg-white were added, and the remaining two tubes were tested in the usual way. The precipitates were treated in the manner above described, and weighed. No precipitates occurred in the secondary superfluids of tubes Nos. 3 and 4.

Table II.

No. of tube.	Weight of dried egg-white.	Amount of anti-serum.	Amount of saline solution.	Weight of precipitate.	Total weight of the precipitates.	Total weight of precipitate from 1 c.c. antiserum.
	milligrammes.	c.c.	c.c.	milligrammes.	milligrammes.	milligrammes.
1	70	3	50	2·8	3·5	1·16
1B	+ 70	—	—	0·7		
2	140	3	50	3·2	3·2	1·06
2B	+140	—	—	trace		
3	280	3	50	3·0	3·0	1·0
4	560	3	50	3·2	3·2	1·06

The superfluids from tubes 1B and 2B were tested for the presence of precipitin by the addition of more protein, but no precipitates were obtained. In this series the weight of precipitate obtained from 1 c.c. antiserum remains constant, despite the large increase in the quantity of protein. No stress can be laid on the amount of precipitate in tube No. 1B, since it is doubtful whether the interaction in tube No. 1 was complete in 24 hours.

As it appears that the precipitate from a given quantity of antiserum is constant, provided there be sufficient protein to neutralise the precipitin, a series of experiments in which the amount of antiserum was varied may be considered. With these experiments may be considered one in which a duplicate was carried out. The details of the experiments were varied to avoid errors. The results are recorded in Table III.

The quantity of protein was found to be sufficient to neutralise all the precipitin except in tubes Nos. 11, 12, and 13. The results show that the

Table III.

No. of tube.	Antiserum.	Amount of antiserum.	Weight of dried protein.	Amount of saline solution.	Weight of precipitate.	Weight of precipitate from 1 c.c. antiserum.
		c.c.	mgrms.	c.c.	mgrms.	mgrms.
1	Horse serum, 57...	2·5	100	50	3·7	1·5
2	„ ...	2·5	100	50	3·5	1·4
3	Hen egg-white, 59	2·0	134	50	8·6	4·3
4	„	3·0	134	50	12·5	4·2
5	„	4·0	134	50	16·7	4·2
6	Horse serum, 53...	2·5	50	50	2·0	0·8
7	„	5·0	200	50	4·0	0·8
8	Horse serum, 56...	5·0	100	50	10·4	2·1
9	„	10·0	100	50	20·0	2·0
10	Hen egg-white, 64	1·0	28	50	1·4	1·4
11	„	2·0	28	50	2·2	1·35
11B	....	—	+140	—	0·5	
12	Hen egg-white, 64	3·0	28	50	3·2	1·4
12B	....	—	+ 56	—	1·0	
13	Hen egg-white, 64	4·0	28	50	3·6	1·5
13B	....	—	+ 56	—	2·4	

amount of precipitate yielded by each antiserum is a fixed quantity for each cubic centimetre of antiserum. It must be noted that the amount of saline solution used as a diluent is the same throughout the series. In tubes Nos. 1 and 2, 2·5 c.c. antiserum for horse serum interacted with 100 milligrammes dried horse serum and the duplicates agree well. In tubes Nos. 3, 4, and 5, 2, 3, and 4 c.c. fowl's egg-white antiserum interacted with 100 milligrammes dried egg-white, yielding 8·6, 12·5, and 16·7 milligrammes precipitate respectively. Calculating the amount for each cubic centimetre antiserum it is found to be about 4·2 milligrammes in each case. In tubes Nos. 6 and 7, 2·5 c.c. antiserum for horse serum reacted with 50 milligrammes horse serum, and 5 c.c. antiserum reacted with 200 milligrammes horse serum. The amount of precipitate in tube No. 7 was double that in tube No. 6. In tubes Nos. 8 and 9, 5 c.c. and 10 c.c. antiserum each interacted with 100 milligrammes dried egg-white and the weights of the precipitates agree sufficiently for each cubic centimetre. In tubes Nos. 10, 11, 12, and 13, 1, 2, 3, and 4 c.c. antiserum for fowl's egg-white each reacted with 28 milligrammes dried egg-white. After 48 hours the superfluids were removed and a quantity of dried egg-white dissolved in saline solution added to each superfluid. No further precipitate formed in tube No. 10, but precipitates formed in tubes Nos. 11B, 12B, and 13B. Here, again, there is fair agreement in the amount of precipitate finally obtained from each cubic centimetre of antiserum.

When the quantity of protein is not sufficient to neutralise all the precipitin in a given amount of antiserum the weight of precipitate is diminished. An experiment showing the relation of the precipitate to the amounts of the interacting bodies may be now described. A rabbit was immunised by the injection of 9·6 grammes dried egg-white in eight doses. The quantities employed and the results obtained are recorded in Table IV.

Table IV.

No. of tube.	Amount of antiserum.	Weight of protein.	Amount of saline solution.	Weight of precipitate.	Weight of precipitate from 1 c.c. antiserum.
	c.c.	milligrammes.	c.c.	milligrammes.	milligrammes.
1	3	1·44	50·0	1·0	0·33
2	3	3·6	50·0	1·5	0·5
3	3	7·2	50·0	2·0	0·66
4	3	14·4	50·0	2·7	0·9
5	3	28·8	50·0	4·2	1·4
6	3	144·0	50·0	6·5	2·2

It will be seen that the amount of antiserum was 3 c.c. in each tube. This quantity yields such small precipitates in the tubes Nos. 1 and 2 that great stress cannot be placed on these figures. The weight of the precipitates has steadily increased. The amount of antiserum obtained from a rabbit is not usually more than 20 c.c., so that extended series cannot be carried out with antisera from rabbits. It was not considered legitimate to employ mixed antisera. Other series gave similar results, but at present the data are too few to discuss these results at length to determine the type of the interaction.

The effect of the degree of dilution on the weight of precipitate may be now considered. Two series of experiments were carried out. In the first series the quantity of egg-white was constant and the amount of saline solution used to dilute the interacting masses was varied. In the second series the concentration of egg-white was maintained constant in the varying amounts of saline solution. The antisera employed were two fowls' egg-white antisera prepared from rabbits. The results are recorded in Table V.

The results of both series correspond, though the absolute amount of precipitate from each antiserum was different. With a quantity of saline solution of 25 c.c. there was a reduction in the weight of precipitate. All observers have noted this reduction, which has been usually ascribed to a solvent action of the concentrated serum. With a quantity of saline solution of 100 c.c. the weights of the precipitates were also slightly reduced. This reduction was probably due to incomplete reaction in 48 hours, since the superfluids removed from tubes Nos. 3 and 6 yielded small precipitates on standing for another 48 hours.

Table V.

No. of tube.	Amount of antiserum.	Weight of egg-white.	Amount of saline solution.	Weight of precipitate.	Weight of precipitate from 1 c.c. antiserum.
	c.c.	milligrammes.	c.c.	milligrammes.	milligrammes.
1	5A	100	25	10·8	2·2
2	5A	100	50	16·2	3·2
3	5A	100	100	15·0	3·0
4	5B	100	25	10·6	2·1
5	5B	200	50	19·5	3·9
6	5B	400	100	17·7	3·6

The results recorded above are typical of those obtained by the examination of 23 different antisera. The amounts of precipitate obtained from the various antisera showed much variation. The quantity of homologous protein required to precipitate completely the precipitin showed similar variations. The gravimetric results confirm the volumetric results of Welsh and Chapman* as to (1) the amount of precipitable substance in an antiserum, and (2) the amount of homologous protein necessary to neutralise and precipitate this precipitable substance. The quantity of precipitate obtained from 1 c.c. antiserum on the complete precipitation of the precipitable substance of the antiserum is recorded in Table VI.†

The results show that the weight of precipitate varied from 0·8 milligramme to 4·3 milligrammes from each cubic centimetre.

Remarks on the Experimental Results.

When an amount of antiserum, *e.g.* 3 c.c., interacts in suitable dilution with increasing quantities of homologous protein, the weight of the precipitate augments as the quantity of homologous protein taking part in the interaction is increased (Table IV). The rate of increase in the weight of the precipitate is rapid as the quantity of homologous protein rises from minute quantities to 5 milligrammes or 10 milligrammes, the exact amount varying with different antisera. With further increments of homologous protein this rate of increase is diminished. When the quantity of homologous protein reaches from 30 milligrammes to 100 milligrammes (the exact amount varying with different antisera) any increase in the weight of the precipitate ceases.

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* 'Journ. of Hygiene,' vol. 6, p. 262, 1906.

† An analysis of the effect of the various factors of the process of immunisation on the weight of the precipitate will be published later.

Table VI.

No. of antiserum.	Nature of protein used for immunisation.	Weight of precipitate from 1 c.c. antiserum.
		milligrammes.
42	Horse serum	1·4
43	,,	
44	Ostrich egg-white	3·8
46	Fowl's egg-white	2·7
47	,, ,,	
48	,, ,,	
49	,, ,,	2·4
50	,, ,,	3·2
51	,, ,,	3·9
52	,, ,,	1·5
53	Horse serum	2·0
54	,,	1·6
55	,,	4·3
56	,,	0·8
57	,,	1·45
58	Fowl's egg-white	4·3
59	,, ,,	4·3
60	,, ,,	3·2
62	,, ,,	2·2
63	,, ,,	1·7
64	,, ,,	1·5
65	,, ,,	1·2
66	,, ,,	1·16

Further increase in the quantity of the homologous protein (in these experiments up to 560 milligrammes protein) leads to no further increase in the weight of the precipitates (Tables I and II). With other amounts of antiserum, such as 1 c.c. or 5 c.c., interacting with increasing quantities of homologous protein, similar phenomena are observed. The weights of the precipitates rise to a maximum and then remain constant.

When the amounts of antiserum are varied, the weights of the precipitates are directly proportional to the amounts of antiserum as soon as the quantities of homologous protein are sufficient to produce the maximal precipitates (Table III). These quantities of homologous protein are those which neutralise completely the precipitin in the antiserum. The superfluids from such interactions fail to yield precipitates on the addition of any quantity of homologous protein. If the quantity of homologous protein be insufficient to neutralise all the precipitin in the antiserum, the weight of the precipitate is diminished and the addition of a further quantity of homologous protein to the superfluid leads to the formation of a precipitate. When the precipitin is neutralised in two or more stages by further increments of homologous protein, the weight of the combined precipitates is equal to that of the precipitate formed in a single interaction with a quantity of homologous protein sufficient to neutralise completely the precipitin (Tables II and III).

The quantities of homologous protein sufficient to neutralise the precipitin in varying amounts of the same antiserum show some relationship to the amount of antiserum. The quantities of homologous protein augment with an increase in the amounts of antiserum, but whether the quantities of homologous protein are directly proportional to the amounts of antiserum is not ascertainable from the present data. The weights of the precipitates formed when increasing amounts of antiserum interact with a constant quantity of homologous protein insufficient to neutralise the whole of the precipitin in the antiserum augment with the amounts of antiserum, but the weights of the precipitates from each unit of antiserum diminish as the amounts of antiserum increase (Table III, tubes Nos. 10, 11, 12, and 13).

The weights of the precipitates from 1 c.c. of different antisera are of value in showing the order of magnitude of the weights of precipitin taking part in precipitin interactions.

In conclusion, I beg to express my indebtedness to Prof. Anderson Stuart, in whose laboratory this research was carried out.

The Influence of Bacterial Endotoxins on Phagocytosis.

(Preliminary Report.)

By Leonard S. Dudgeon, P. N. Panton and H. A. F. Wilson.

(Communicated by Dr. F. W. Mott, F.R.S. Received April 26,—Read June 2, 1910.)

(From the Pathological Laboratories, St. Thomas's Hospital.)

These investigations were undertaken for the purpose of determining the effect of endotoxic substances on phagocytosis, as tested *in vitro*. Experiments were made to determine whether these substances, when added to a phagocytic mixture, would cause an increase or a decrease in the phagocytic activity; whether such action would be general or specific; whether the action would be affected by subjecting the endotoxins to varying degrees of temperature, and whether the toxins would act directly on the bacteria, the serum, or the leucocytes.

The present communication is intended only for the purpose of introducing our preliminary results, which are derived from a considerable amount of experimental enquiry. The explanation of the mode by which the effects to be described are produced is now under investigation.

Technique.—The organisms used in these experiments were as follows:—*B. Typhosus, B. Paratyphosus, B. Achard, B. Danysz, B. Coli* (several strains), *B. Friedlander, B. Proteus, B. Prodigiosus, B. Pyocyaneus, Micrococcus Aureus.* These were cultivated on agar at 37° C., and on gelatin at 22° C., for 24 to 72 hours. In one instance certain of the bacteria referred to above were obtained from broth flasks, which had been incubated for three weeks at 37° C. The bacterial extracts were made originally from cultures grown on the surface of the agar, but, for reasons which will be referred to subsequently, this medium was abandoned for gelatin.

To a sufficient growth of the organisms on plate cultivations a measured quantity of sterile salt solution was added, the entire growth was carefully removed, and the thick suspension of bacteria in saline transferred to a sterile agate mortar. The organisms were then ground up with considerable force in the presence of sterile powdered glass, or, in our later experiments, of sand. The bacterial extract was then transferred to sterile tubes, centrifugalised at high speed, and the supernatant fluid pipetted off into fresh tubes; this process was repeated until an extract was obtained free from bacteria. It was noted that the extracts, and more particularly those obtained from *B. Pyocyaneus* and *B. Proteus*, were often turbid, and that the turbidity was increased when the extracts were subjected to high temperatures. It was undesirable in the case of certain bacteria, namely, *B. Typhosus, B. Achard* and *B. Paratyphosus*, to obtain the extracts from the living organisms; in these instances the cultures were first killed by exposure to heat in the usual way, but similar results were obtained whether the extract was prepared from living bacteria or from those killed by heat. In a few instances the bacterial suspension was ground up after being frozen in an agate mortar surrounded by solid CO_2. The phagocytic mixture consisted of washed human leucocytes, pooled normal sera and 24 hour old suspensions in saline of living, never of heated, bacteria. The whole was incubated for 15 minutes at 37° C. In every case fifty leucocytes were counted with the number of bacteria engulfed, and the ratio of phagocytic to non-phagocytic cells noted.

It is convenient to mention here that extracts of organisms cultivated on agar were abandoned; because, in the majority of instances, the agar medium itself was found to directly inhibit phagocytosis. We employed agar media standardised to $+1{\cdot}5$, $+1{\cdot}0$, and $+0{\cdot}5$ made in our laboratories, and also that derived from other institutions, but similar results were obtained in all cases; when gelatin was substituted for agar the results were constant, and free from error.

An essential point in the technique, and one upon which the most satisfactory results depend, is to procure a bacterial extract as concentrated as possible. In our earlier experiments one part of bacterial extract was mixed with two parts of normal serum and either incubated for one hour at 37° C., and then one volume of the mixture added to equal volumes of leucocytes and bacteria, or the extract was added after 15 minutes incubation. It was found, however, that the best results were obtained by incubating for one hour at 37° C. equal volumes of serum and bacterial extract, and then mixing equal volumes of this with the leucocytes and bacteria. Incubation for 15 minutes failed to produce the striking effects shown when longer incubation was employed.

The Action of the Endotoxic Substance on the Leucocytes.

Owing to the limited number of experiments performed, this part of our investigation is incomplete. It would appear that if the endotoxin acts directly on the leucocytes at all, they are capable of complete recovery during the process of washing. Our experiments fail to indicate any such direct action on the leucocytes themselves.

The Action of the Endotoxic Substance on the Serum.

Equal parts of serum and bacterial extract were mixed together and incubated for one hour at 37° C. One volume of this mixture was then added to equal volumes of washed leucocytes and the bacterial suspension. The experiments were completed in the usual way. In every instance equal volumes of serum and normal saline were mixed and incubated for the same time, and used as a control.

The following experiments show the direct action of the endotoxin and serum mixture.

Experiment 1.*

A. Serum saline mixture + leucocytes + *B. pyocyaneus*—
50 cells contained 262 bacilli; non-phagocytic cells, 0.

B. Serum pyocyaneus extract + leucocytes + *B. pyocyaneus*—
50 cells contained 41 bacilli; non-phagocytic cells, 30.

Experiment 2.

A. Serum saline + leucocytes + *B. pyocyaneus*—
50 cells contained 390 bacilli; non-phagocytic cells, 0.

B. Serum pyocyaneus extract + leucocytes + *B. pyocyaneus*—
50 cells contained 12 bacilli; non-phagocytic cells, 43.

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* In this and the following experiments by "serum saline," "serum pyocyaneus extract," etc., is meant one volume of a mixture of equal parts of serum and salt solution, or of serum and the bacterial extract in question, after one hour's incubation at 37° C.

Experiment 3.

A. Serum saline + leucocytes + *B. proteus*—
50 cells contained 474 bacilli; non-phagocytic cells, 0.

B. Serum proteus extract + leucocytes + *B. proteus*—
50 cells contained 33 bacilli; non-phagocytic cells, 24.

The following experiments demonstrate the completely specific action of the endotoxin:—

	No. of bacteria in 50 cells.	No. of non-phagocytic cells.
Experiment 1.		
A. Serum saline + leucocytes + *B. paratyphosus B.*	192	0
,, + ,, + *B. Danysz*	62	16
B. Serum *paratyphosus B.* extract + leucocytes + *B. paratyphosus B.*	15	39
,, ,, ,, + leucocytes + *B. Danysz*	89	13
Experiment 2.		
A. Serum saline + leucocytes + *B. Achard*	249	1
,, + ,, + *B. Danysz*	268	1
B. Serum *B. Danysz* extract + leucocytes + *B. Achard*	102	11
,, ,, ,, + ,, + *B. Danysz*	25	33
C. Serum *B. Achard* extract + leucocytes + *B. Achard*	10	42
,, ,, ,, + ,, + *B. Danysz*	123	11
D. Serum *B. coli* extract + leucocytes + *B. Achard*	156	2
,, ,, ,, + ,, + *B. Danysz*	183	3
Experiment 3.		
A. Serum saline + leucocytes + *B. pyocyaneus*	234	0
,, + ,, + *M. aureus*	99	20
B. Serum *B. pyocyaneus* extract + leucocytes + *B. pyocyaneus*	7	44
,, ,, + ,, + *M. aureus*	56	24
,, ,, + ,, + *B. pyocyaneus*	equal vols. 7	44
,, ,, + ,, + *M. aureus*	36	36
C. Serum *M. aureus* extract + leucocytes + *B. pyocyaneus*	236	4
,, ,, + ,, + *M. aureus*	36	38
,, ,, + ,, + *B. pyocyaneus*	167	6
,, ,, + ,, + *M. aureus*	53	28

In the third experiment of this series, the specific nature of the endotoxic action is clearly shown when the leucocytes and equal volumes of cocci and bacilli are mixed together.

We may remark that the degree of phagocytosis in all our experiments is estimated by the number of organisms engulfed in a given number of cells, but the ratio of active and non-active phagocytes is at least of equal importance. In these observations attention has been paid to the percentage of non-phagocytic cells, the importance of which has been previously insisted upon by many workers on phagocytosis.

When the reduction of phagocytosis by endotoxins had been proved,* investigations were undertaken for the purpose of ascertaining whether these toxic substances were resistant to heat. It was found that they were unaffected by exposure to a temperature of 60° C. for periods varying from 15 minutes to three hours, and that the results obtained with the heated substance were identical with those obtained with the unheated, as may be seen from the following experiment.

	No. of bacteria in 50 cells.	No. of non-phagocytic cells.
A. Serum saline mixture + leucocytes + *M. aureus*	182	9
„ „ + „ + *B. typhosus*	198	3
B. Serum *B. typhosus* extract + leucocytes + *M. aureus*	26	43
„ „ + „ + *B. typhosus*	17	38
C. Serum *B. typhosus* extract* + leucocytes + *M. aureus*	46	33
„ „ + „ + *B. typhosus*	15	37

* This extract has been exposed to a temperature of 60° C. for three hours.

The Effect of Diluting the Endotoxin.

The effect of diluting an extract before mixing it with the serum allowed a return to the same degree of phagocytosis as occurred in the serum saline mixture, *i.e.*, the control. Several notable exceptions were recorded, in which the diluted toxic substance appeared to play the part of a "stimulin." In such instances the degree of phagocytosis was far greater than in the control experiments. The stimulating action of the diluted toxin appeared to be specific. This may be briefly instanced by the following examples:—

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* Prof. Hewlett very kindly suggested to us that we should ascertain whether similar results could be obtained by substituting *washed* bacteria for the preparation of the endotoxin, so as to eliminate the possibility that our results might be dependent upon an extracellular substance adhering to the unwashed organisms. Experiments were performed for this purpose and it would seem that washed bacteria can be employed for the preparation of the bacterial extract, although the results are not quite so striking as in the case of the unwashed. This fact could be explained possibly by the loss of bacteria in the washing, and, therefore, the small quantity of organisms left for grinding up.

	No. of bacteria in 50 cells.	No. of non-phagocytic cells.
Experiment 1.		
A. Serum saline + normal leucocytes + *B. paratyphosus B.* ...	230	2
B. Serum bacterial extract from *B. paratyphosus B.* + normal leucocytes + *B. paratyphosus B.*	32	23
C. Serum bacterial extract from *B. paratyphosus B.* diluted 1/10 + normal leucocytes + *B. paratyphosus B.*	492	0
Experiment 2.		
A. Serum saline + normal leucocytes + *B. prodigiosus*............	117	5
„ + *B. typhosus*	181	9
B. Serum bacterial extract from *B. typhosus* diluted 1/10 + normal leucocytes + *B. prodigiosus*	153	4
Serum bacterial extract from *B. typhosus* diluted 1/10 + normal leucocytes + *B. typhosus*	372	0

A Case of Sleeping Sickness Studied by Precise Enumerative Methods: Regular Periodical Increase of the Parasites Disclosed.

By Major Ronald Ross, F.R.S., and David Thomson, M.B., Ch.B., D.P.H.

(Received May 2,—Read June 16, 1910.)

Prefatory Note by R. Ross.—For a long time it has appeared to me that much light might be thrown on infectious diseases, immunity and treatment, by more exact enumeration of the infecting organisms, and that we might even be able ultimately to apply mathematical reasoning to the study of these subjects. In 1903* I elaborated a method of blood examination, called the *thick-film process*, which enables us to detect small organisms in the blood about twenty times more easily than in ordinary preparations; but for the lack of the necessary assistance I was long unable to apply the method to the laborious enumeration of such organisms. Recently, however, the Advisory Committee for the Tropical Diseases' Research Fund has placed considerable funds at the disposal of the Liverpool School of Tropical Medicine for the study of cases in the tropical ward of the Royal Southern

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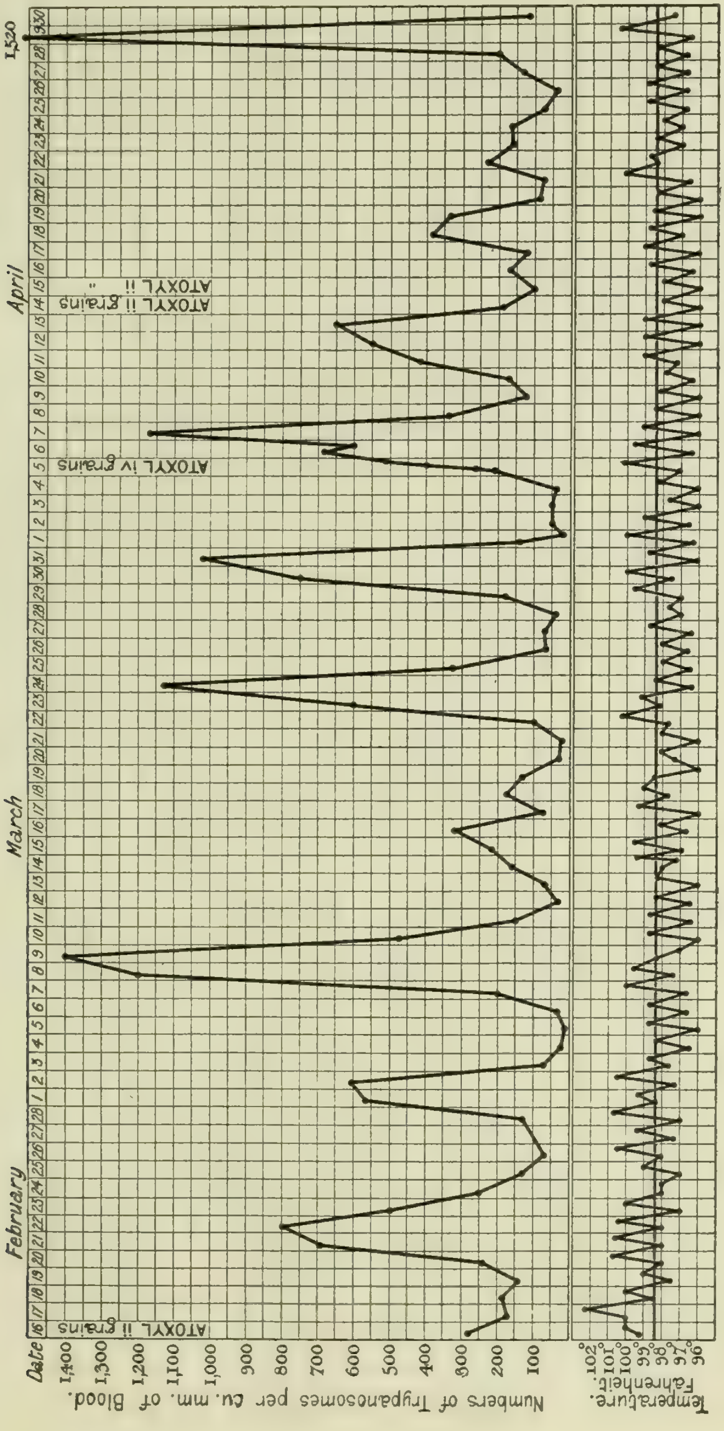
* 'Lancet,' January 10, 1903, and Thompson Yates Reports, vol. 5, Part I, 1903.

Hospital, Liverpool—with the result that the investigations referred to were commenced by Dr. David Thomson and myself from the beginning of this year. As I expected, methodical counting of the parasites has at once verified or disclosed several facts of importance in connection with malaria and trypanosomiasis. We now limit ourselves to a brief description of the remarkable periodical increase of *Trypanosoma gambiense* revealed by careful daily counting in a case in my clinic (the case, which is still under treatment, will be fully reported elsewhere).

W. A., male, aged 26 years, a strong young man born in Northumberland, was infected in N.E. Rhodesia in September, 1909, the trypanosomes being found in his blood in Africa on November 17. On coming to Liverpool for treatment he was admitted into the Southern Hospital on December 4. From then until February 16 (73 days) the number of trypanosomes in his blood was estimated only by the rough methods in common use; that is, by the proportion of trypanosomes to red cells or leucocytes, or to "fields" of the microscope examined. These methods are obviously open to such great error that they can scarcely be depended upon to indicate any but very large differences in the numbers of objects counted. During the 73 days 46 counts were made; but on several occasions none was attempted for three or four days in succession—so that, even if the methods of counting employed had been more accurate, sudden fluctuations might easily have been missed. Hence, as was to be expected, the graph during this period is very irregular and almost worthless. On admission on December 4 the patient was reported to contain about 6000 trypanosomes per cubic millimetre of blood, and large numbers, amounting to about 3000 per cubic millimetre, were found on December 17 and 28 and on January 16. All this time the patient was given the usual treatment with atoxyl and mercury, and received altogether 10 doses of two to four grains of the former. Nevertheless the parasites never fell below about 200 per cubic millimetre in number, as roughly estimated.

It was then found, however, that atoxyl was injuring the patient's sight (as sometimes happens), and other treatment was substituted. At the same time we elaborated a much more correct method of counting all the parasites in measured quantities (one quarter to 1 cubic millimetre) of blood taken in thick film; and from February 16 onward the patient's trypanosomes were estimated daily by this method by one of us (D. T.). The attached chart gives the remarkable graph obtained up to the present (April 30).

The numbers of trypanosomes found were scrupulously recorded, and the


Date
February
March
April
1,520
ATOXYL ii grains
ATOXYL iv grains
ATOXYL ii grains
ATOXYL ii "
1,400
1,300
1,200
1,100
1,000
900
800
700
600
500
400
300
200
100
Numbers of Trypanosomes per cu. mm. of Blood.
102°
101°
100°
99°
98°
97°
96°
Temperature. Fahrenheit.

smoothness and regularity of the graph suggest that there was no very great error of observation. The blood was taken every day at about 10 A.M., but on April 5 and 6 several counts were made daily.

It will be seen that between February 16 and April 30 (73 days) there were 11 rises in the number of the parasites. Up to April 7 there were seven rises, at intervals of seven or eight days. During this period the patient was given no atoxyl, but was treated with large doses of quinine (30 to 40 grains) daily, with frequent doses of methylene blue, and with trypan red on March 17, 18, 19, and 20.

On April 5 it was decided to administer atoxyl again as shown on the chart, together with mercury and other treatment.

The temperatures were taken by the sister of the ward, and it will be seen that there has always been a tendency to a slight rise in temperature concurrent with the rise in the number of parasites—the two curves thus confirming each other. Only the maximum and minimum temperatures are entered in the accompanying chart.

The great regularity of the rises can scarcely be compatible with a mere chance distribution. It will also be observed that the rises were of two kinds, namely, high rises and low rises; and that the two kinds alternated with regular periodicity until April 18, at which point the cycle appears to have become distorted—probably in consequence of the treatment. The regularity of alternation of the high and low rises is so well marked as to recall the picture of a double tertian malaria, and to suggest that two independent sets of parasites may exist in the patient, just as often happens in malaria.

The large dose of atoxyl given on April 5 seems to have had no effect whatever on the following rise; but the succeeding rises were apparently modified for some reason. The value of the enumerative method for therapeutical research is obvious.

Of course, many other facts in connection with the case have been recorded, and parallel work is being done on sub-inoculated animals and on the parasites. It is, therefore, inadvisable to attempt at present any discussion of the many interesting theoretical questions which arise.

We are much indebted to the Director of the Sleeping Sickness Bureau (Dr. Bagshawe) for having given us references to the literature on the subject of such fluctuations. In the original case of Dutton and Ford it was noted that the parasites varied in numbers, and that a parallel rise in the patient's temperature occurred. Manson and Daniels* chart the number of parasites compared with 500 leucocytes; but the error of this method is very large and their graph is quite irregular. They abandoned counts in measured quantities of blood as "unreliable." Thomas and Breinl* showed that in three cases of Sleeping Sickness the numbers of trypanosomes found in "fresh cover-slip preparations" varied irregularly from time to time. Koch, Beck, and Kleine (1909) remark on the irregularity of the appearance of *T. gambiense* in African natives, and state the parasites are present for two to five days and absent for two to three weeks. Salvin-Moore and Breinl† show a graph with two undulations and a final premortal rise in two heavily infected rats, and give a detailed description of corresponding changes in the parasites. Apparently, hitherto, only irregular variations in the numbers of the parasites seem to have been recognised; probably the large error due to inadequate methods of counting has disguised the regular periodicity of the variation shown by more exact counts in the 11 successive undulations observed in our case.

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* 'British Medical Journal,' May 30, 1903.

We should add that our methods enable us to detect parasites when they are in numbers so small that their detection by the ordinary methods would be exceedingly laborious. Hence if our case had been studied by the ordinary methods, probably only the crests of the rises would have been visible in the chart, and it would have been said that the parasites had disappeared in the intervals.

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* 'Memoirs of the Liverpool School of Tropical Medicine,' vol. 16, 1905.

† 'Annals of Tropical Medicine, Liverpool,' vol. 1, No. 3, 1907.

On the Recognition of the Individual by Hæmolytic Methods. (*Preliminary Communication.*)

By Charles Todd, M.D., Bacteriologist, Egyptian Government, and
R. G. White, M.B., Director, Serum Institute, Cairo.

(Communicated by Dr. C. J. Martin, F.R.S. Received May 5,—Read June 16, 1910.)

(From the Hygienic Institute, Public Health Department, Cairo.)

The following is a brief account of some results obtained during the course of an investigation into the artificially produced hæmolytic isolysins of the ox.

The peculiar interest attaching to this class of bodies was first pointed out by Ehrlich and Morgenroth in their classical studies on hæmolysis, where they showed by the treatment of goats with large quantities of laked goats' blood, that hæmolytic isolysins were formed in the blood of the immunised animals. Their investigations were made with sera prepared in this way.

The isolysins so obtained were, however, somewhat weak: in the most favourable case 0·3 c.c. of serum being required to hæmolyse 1 c.c. of 5 per cent. suspension of red blood corpuscles.

It occurred to us that it might be interesting to examine the serum of the cattle used for the production of Rinderpest serum at the Serum Institute in Cairo, as these animals would appear to be under ideal conditions for the formation of isolysins. We had at our disposal about 100 Egyptian cattle immunised against Rinderpest by Kolle and Turner's method which consists in a preliminary simultaneous inoculation of a small quantity of virulent blood and a suitable quantity of serum, followed after a time, by a massive dose of 4 litres of virulent cattle blood given intramuscularly and repeated at intervals of about two months.

The results of the investigation of the blood of these animals are being published separately and need not be gone into in any detail here, but we may state that they entirely agree with the results obtained by Ehrlich and Morgenroth in the case of the isolysins for the goat. We were, however, fortunate in having at our disposal a large number of animals yielding powerful sera.

The fresh serum of the immune cattle when tested on normal ox corpuscles shows little or no hæmolytic action, but if a small quantity of fresh guinea-pig serum is added, hæmolysis takes place very rapidly.

It was thus found that the serum of almost all the immune cattle, in the presence of a suitable complement, was highly hæmolytic, the hæmolytic power naturally depending on the amount of blood which the animal had received and on the date on which it was bled.

The hæmolytic power of all the animals in the Institute was examined. The sera of 76 out of a total of 101 were found to be capable of causing the complete hæmolysis of 1 c.c. of a 5 per cent. suspension of the corpuscles of cattle imported from Cyprus, in one hour at 37° C., in as small an amount as one-hundredth of a cubic centimetre in the presence of a suitable amount of guinea-pig complement, and many sera hæmolysed at much smaller doses.

If the serum of one immune animal is tested on the corpuscles of a number of individual animals of the same species, a great variation is seen in its hæmolytic action on the various corpuscles; some being very highly susceptible, others less so, and others almost unaffected.*

If now the serum of a second immune animal is tested on the same series of corpuscles, we get again differences in the action on the various corpuscles, and again certain corpuscles are picked out as susceptible, while others are less affected.

The two sera, however, do not necessarily pick out the corpuscles of the same individuals, so that corpuscles which may be highly sensitive to one serum may be almost unaffected by another.

It is a curious fact that, so far as we have seen, the race of the animal appears to be more or less immaterial and any influence exerted by this is quite masked by the individual characteristics.

In no case was the serum of an immunised animal found to be hæmolytic for its own red blood corpuscles, so that we were dealing with an iso-, not an auto-lysin. This result agrees with what Ehrlich and Morgenroth found in the case of their goats.

The complement of the ox, as occurring in the fresh immune serum, even in the presence of a powerful hæmolytic isolysin, appears to be capable of effecting hæmolysis of ox corpuscles only to a very slight extent or not at all.

If we imagine that the formation of these hæmolytic isolysins in the ox is protective, and that it is an attempt on the part of the organism to effect the solution and removal of the red blood corpuscles which have been introduced into the body, it would appear improbable that the organism should, so to speak, go to the trouble of elaborating a hæmolytic amboceptor which is practically useless for the only complement with which it is likely to come into contact, and it would seem probable that a suitable complement is really available somewhere in the body although not obviously present to any extent in the serum.

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* In this connection we have just seen a paper in the latest number of the 'Muenchener Medizinische Wochenschrift' (April 5, 1910) by v. Dungern and Hirschfeld, who, working with agglutinins, have, by a somewhat similar method, shown that it is possible to divide up the animals of one species into classes, according to their agglutinating reactions.

With a view to seeing if this is really the case, a normal Cyprus bull was injected intravenously with one litre of the mixed serum of ten immune cattle. This serum, although only 24 hours old, showed no action on ox corpuscles "in vitro" if no foreign complement was added; but in the presence of fresh guinea-pig serum was very powerfully hæmolytic. (0·01 c.c. being sufficient to hæmolyse 1 c.c. of 5 per cent. suspension of ox corpuscles.) A few hours after the injection, the urine was very darkly hæmoglobin stained, showing that a suitable complement had been forthcoming.

On testing the serum of the animal a few days later, it was found that, although it had now no hæmolytic action on its own corpuscles, it was distinctly hæmolytic for the corpuscles of many other individuals. It was thus possible to "exhaust" the immune serum for one particular corpuscle and to leave it still hæmolytic for many others.

This method of exhaustion "in vivo" was then replaced by exhaustion "in vitro." The technique being as follows:—

The immune serum was mixed with an equal volume of the washed corpuscles with which it was desired to exhaust the serum; the mixture kept at 37° C. for an hour, centrifuged, and the serum again treated in the same way with the washed corpuscles and the process repeated a third time. It was then found that the serum had lost all traces of hæmolytic power for the corpuscles in question.

By means of this method the sera of different immune cattle were now exhausted with the corpuscles of various individuals of the same species and the hæmolytic power of these sera after such treatment was studied on the corpuscles of different individuals.

It was found that if an immune serum is exhausted with corpuscles of an individual (A), it remains hæmolytic for the corpuscles of many other individuals, but loses its hæmolytic power for the corpuscles of some other individuals as well as for those of (A).

If now a second immune serum is exhausted with the same corpuscles (A), its action on the various corpuscles is not exactly parallel to that of the first serum, and often shows very marked differences.

This result is to be expected, as it was shown by Ehrlich and Morgenroth, that two goats each injected with similar doses of the same goat's blood at the same times, gave quite different isolysins. In fact the isolysins formed depend upon two distinct factors:—

(*a*) The individuality of the injected corpuscles.
(*b*) The individuality of the animal into which they are injected.

When we consider the enormous number of variations possible in each of these factors, we see the almost unlimited possibilities in the resulting sera.

In view of the above it should be possible by taking a mixture of a sufficiently large number of immune sera and exhausting this with the corpuscles of one individual, to obtain a serum which is specific for the corpuscles of this one individual; *i.e.* which has no hæmolytic action on these corpuscles, but hæmolyses those of all other individuals of the same species. To test this, a mixture was made of the sera of between 60 and 70 immune Egyptian cattle. This mixture was then exhausted with the corpuscles of a normal Cyprus bull and then tested on the washed corpuscles of 20 immune Egyptian cattle, two normal Cyprus cattle, and the above mentioned Cyprus bull with whose corpuscles the mixture had been exhausted. For the test equal parts were taken of—

(*a*) The exhausted serum;
(*b*) A 5 per cent. suspension of the washed red blood corpuscles;
(*c*) A one-tenth dilution of fresh guinea-pig serum in normal saline.

The tubes were kept at 37° C. for one hour and then left over-night in the ice-safe; after which the results were read off.

It was then found that complete hæmolysis had occurred in all the tubes, with the exception of the control tube containing corpuscles of the Cyprus bull with which the serum was exhausted. The "exhausted" serum was thus able to pick out, quite sharply, the corpuscles of one individual from those of 22 others. Following up these results, a second and more extensive test was made. In this case the same immune serum was used; it was, however, exhausted with the corpuscles of another normal bull.

This exhausted serum was then tested on the corpuscles of 110 different cattle (3 Soudan, 34 Cyprus and 73 Egyptian).

In this test again all the tubes showed complete hæmolysis with the exception of the one containing the corpuscles for which the serum had been "exhausted." This control tube showed no trace of hæmolysis.

A number of other tests have been made by exhausting the serum with the corpuscles of various individuals, and the general rule has so far always held except in one case. The serum exhausted with the corpuscles of a cow was found to have lost its hæmolytic action, not only for the corpuscles of the cow, but also for those of its calf.

It is worth noting, however, that the serum exhausted with the corpuscles of the calf was specific for the calf.

Taking advantage of the fact that the isolysins for the ox act very powerfully on the corpuscles of the goat, we have made a series of experiments to test the utility of the method in investigating the corpuscles of closely related individuals. The method works well and is giving very interesting results.

These results show that the red blood cells of different individuals of the same species are not only not identical, but that they are characterised by a definite individuality and can be distinguished from the red blood cells of any other individual.

How far this is part of a general law affecting the other cells of the body is at present under investigation.

We are of the opinion that the method of investigation by means of exhausted polyvalent immune sera will prove most valuable in biological research not only as a means of identifying the cells of the individual, but in the investigation of the laws of heredity and many other important problems.

Conclusions.

1. The immunisation of the ox with the red blood corpuscles of other oxen gives rise to the formation of a hæmolytic amboceptor in the blood of the immunised animals.

2. The amboceptor so formed is an *isolysin* but not an *autolysin.*

3. The race of the animal appears to have very little influence on the resulting hæmolysins.

4. The serum of an animal so treated acts very differently on the red blood corpuscles of different individual oxen.

5. The sera of different individuals similarly immunised differ from one another in their action on the corpuscles of different individuals.

6. If the serum of a single immunised animal be "exhausted" with excess of the corpuscles of one other individual, the serum loses its power of hæmolysing the corpuscles of this individual, while retaining the power of hæmolysing the corpuscles of many, but not all, other individuals.

7. If, however, a polyvalent serum be made by mixing the sera of a large number of immunised animals, and this serum is exhausted with the corpuscles of any one individual, the serum entirely loses its power of hæmolysing the corpuscles of this individual, but remains strongly hæmolytic

for all other individuals not closely related to the individual whose corpuscles were employed for the exhaustion of the serum.

(N.B.—It is possible that exceptions may be found, but these have not yet been met with except in the case of close blood-relations.)

8. The red blood corpuscles of any individual are thus characterised by a definite individuality of their own, and can be distinguished from those of any other individual of the same species.

Experimental Researches on Vegetable Assimilation and Respiration. VI.—Some Experiments on Assimilation in the Open Air.

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(Communicated by Dr. F. F. Blackman, F.R.S. Received March 1,—Read June 16, 1910.)

CONTENTS.

PAGE
Section I.—Introduction 421
II.—On the Rate of Assimilation of Carbon Dioxide by Leaves of *Helianthus annuus* in Bright Sunshine 422
i. Experiments........ 422
ii. Discussion of Results 430
iii. Brown and Escombe's Experiments with *H. annuus*... 433
III.—Experiments with *Catalpa bignonioides*........ 435
IV.—On the Rate of Assimilation in Nature, and on the Occurrence of Translocation during the day 439
V.—Summary........ 447

Section I.—INTRODUCTION.

In investigating assimilation under natural conditions, gasometric methods, which involve enclosing leaves, are unsuitable. The only method which is free from this objection is the half-leaf dry-weight method introduced by Sachs.[*] Unfortunately, owing to the overlooking of certain grave errors to which this method is liable, most of the earlier work is of uncertain value. Having, however, made a full investigation into the sources and magnitude of these errors, an account of which was published in an earlier paper,[†] I have been able, after introducing appropriate modifications, to use the method with precision, and to obtain results whose degree of approximation to the truth can be justly gauged.

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[*] 'Arbeit. d. Bot. Inst. in Würzburg,' III, 1883, p. 19.

[†] 'Roy. Soc. Proc.,' 1909, B, vol. 82, p. 1.

The vexed question of the highest rate of assimilation possible to detached leaves of *Helianthus annuus* in the open air first claimed attention. Measurements of the gain of dry weight by these leaves on bright sunny days (described in Section II) have clearly proved that Sachs' original value was not excessive, and suggest some of the conditions which must be fulfilled if such rapid assimilation is to be possible.

Numbers for the dry-weight gain in *Catalpa bignonioides* have also been determined, and form a valuable contrast with those for *Helianthus.*

Attention has been paid throughout to the part played by the stomata in regulating the rate of assimilation; and in Section IV are discussed the connected questions as to how far the numbers obtained with detached leaves represent what is occurring with leaves on the plant, and whether any translocation of the accumulating products of photosynthesis takes place during the day.

Section II.—On the Rate of Assimilation of Carbon Dioxide by Leaves of *Helianthus annuus* in Bright Sunshine.

(i) The experiments described in this section were undertaken with the object of checking Sachs' experiment with detached leaves of *Helianthus annuus,* in which he obtained the high value 16·5 milligrammes per square decimetre (1·648 grammes per square metre) per hour as the rate of increase of dry weight on a bright sunny day. Brown* and Morris,† in a similar experiment, found the much smaller rate of increase of 10·0 milligrammes; and Brown and Escombe,‡ determining the intake of carbon dioxide by direct measurement, failed to obtain a higher rate of assimilation in their experimental glass cases than was equivalent to a gain of about 5·5 milligrammes of dry substance per square decimetre per hour.

Notwithstanding this lack of confirmation of Sachs' result, a study of the details of his procedure in this experiment§ leaves no ground for assuming that any known kind of error entered to vitiate it seriously. He guarded against the only source of error which might have made the result far too high, the shrinkage in area of the experimental half-leaves, by floating them on water at the end of the experiment; it is highly probable that he thereby approximately eliminated errors from shrinkage.

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* *Loc. cit.*, p. 25.

† 'Journ. Chem. Soc.,' 1893, pp. 626—8.

‡ 'Roy. Soc. Proc.,' 1905, B, vol. 76, p. 44, Table I.

§ Thoday, D., *loc. cit.*, p. 32.

In the following experiments, it will be observed, leaves which have remained turgid have given results which entirely confirm Sachs' high value. Others have shown smaller rates of increase in proportion to their departure from this ideal condition. Observations with the horn hygroscope* have indicated that the stomatal aperture is at any rate the principal factor in determining the reduction of assimilation.

The experiments were all made with cut leaves. The method of supplying them with water calls for some remark, as much difficulty was met with in keeping them turgid.

The Treatment of Detached Leaves.—Leaves which are really robust, when detached in the early morning, can stand insolation without wilting when the cut leaf-stalk is simply immersed in air-free water. This procedure sufficed to keep fully turgid three of the five leaves used in Expt. 1 in 1908. But in 1909 it was found necessary to supply the less robust leaves that the poor summer produced with water under pressure during insolation. This was done by fastening the leaves, after removing the greater part of their petioles, with rubber tubing to the short arms of J-tubes. The joint was made by wrapping the stalk with cotton wool which had been dipped in melted soft wax-mixture, and tightly tying the rubber tube round this with string. In this way injurious local pressure of the string was avoided. In Expts. 4 and 5 several leaves were attached to the same reservoir of air-free water, 1½ metre above them.

In the first experiment pieces were cut from the control and experimental half-leaves by means of templets. The experimental half-leaves were marked and their change of dimensions during the experiment measured, and a corresponding correction was made for the change of area which had taken place.†

Having thus eliminated the error from change of area there still remains an error from want of identity in respect of dry weight between similar pieces from either side of the midrib of a leaf (the "asymmetry error"). This is an error inherent in the method. Its effect on the result varies with the weight of the leaf and the duration of the experiment, and must, therefore, be estimated for each experiment.‡ The average and maximum errors to be expected from this source are given above the table.

Experiment 1.—August 11, 1908. Five leaves cut 6 A.M., and put in a dull light. Experimental half-leaves set up in the open at 10 A.M.; transferred to the greenhouse at noon because of a storm of wind and rain. Morning, sunshine intermittent; afternoon, sunny; maximum sun temperature,§

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* See p. 429.

† Thoday, *loc. cit.*, p. 37.

‡ *Ibid.*, p. 55; Summary, 5, etc.

§ Given by a bright, bulb mercury thermometer.

25° C.; minimum, 18° C. Experiment closed at 3 P.M.; duration, five hours.

Table I.—Asymmetry error per sq. decim. per hour: average about ± 1·6 milligrammes; maximum, about ± 3 milligrammes.

a = control half-leaf; *b* = experimental half-leaf.

Leaf.	Area, in sq. cm.	Dry weight, in grammes.	Estimated percentage change of area of experimental half-leaf.	Corrected dry weight, in grammes.	Increase of dry weight, in grammes.	Gain per hour per sq. decim., in milligrammes.	Condition of leaf during experiment.
1	(*a*) 60	0·2653	—	0·2653			
	(*b*) 60	0·3130	+1·2	0·3168	0·0515	17·2	Turgid.
2	(*a*) 60	0·2362	—	0·2362			
	(*b*) 60	0·2744	+1·2	0·2777	0·0415	13·8	"
3	(*a*) 70	0·2705	—	0·2705			
	(*b*) 70	0·3245	+0·9	0·3274	0·0569	16·3	"
4	(*a*) 40	0·1652	—	0·1652			
	(*b*) 40	0·1832	+1·0	0·1850	0·0198	9·9	Limp.
5	(*a*) 75	0·3333	—	0·3333			
	(*b*) 75	0·3753	−7·7	0·3463	0·0130	3·5	Wilted.

The average of the rates of increase shown by the turgid leaves is 15·8 milligrammes, which approaches very nearly to Sachs' result, 16·5 milligrammes. It is to be noted that the latter was obtained during a period of unbroken sunshine, in the open air; the conditions under which my experiment was carried out were less favourable and less uniform.

The other two leaves, 4 and 5, gave much smaller results. Leaf 4 had been a little limp throughout the experiment, and only recovered its turgor when it was taken into the laboratory. It showed a rate of increase of 10 milligrammes per square decimetre per hour.

Leaf 5 was quite flaccid throughout the experiment and shrank in area to the extent of about 8 per cent. Its stomata must have closed almost completely soon after it was taken into the open air, and it increased in weight at the relatively small rate of 3·5 milligrammes per square decimetre per hour.

Subsequent sets of experiments all gave similar results.

In these later experiments of 1909 the templet method employed in Expt. 1 was superseded by the stamping method, whereby errors from change of area are completely eliminated* and laborious corrections avoided.

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* Thoday, *loc. cit.*, p. 44.

This method was not complete in all its details when my earlier paper was written, and must now be described more fully than was possible at that time.

(1) *The Stamping Method.*

In this method equal areas are marked out at the beginning of the experiment on both the control and the experimental half-leaves, by means of an inked rubber stamp. Thus the two corresponding areas are comparable, whatever subsequent changes take place in the area of the experimental half-leaf.

The Stamp.—I have used stamps of special design cast in rubber in the same way as ordinary endorsing stamps. Each makes an impression of a rectangle bounded by lines as fine and straight as possible. Surrounding this rectangle closely is another, bounded by lines about a millimetre in thickness; the broad ridge which makes this outer impression serves the purpose of relieving the fine inner ridge from pressure, which would otherwise quickly destroy it. Fig. 1 is a facsimile of an impression made by the smallest stamp which I have used. The inner rectangle measures roughly 2 cm. by 5 cm.

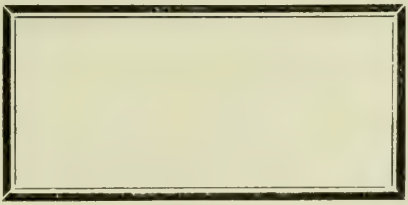

Fig. 1.

Besides this stamp I have used two others, making impressions which measure roughly 4 cm. by 5 cm. and 8 cm. by 5 cm. respectively.

The Mount.—The smallest stamp was mounted on a slip of plate glass, and a cork attached to the other side of the glass as a handle. The other two were obtained mounted like ordinary endorsing stamps.* Ordinary mounting is fairly satisfactory in practice, even for so large an area as 40 sq. cm.; but, for larger areas, it would be advisable to mount the stamp in such a way that the leaf could be clamped within the rectangle to be marked out, before the rubber stamp itself, which would be mounted on a rectangular framework, is brought down upon it.

The Support.—Below the leaf I use a small rectangular piece of board, not much larger than the stamp, covered with velveteen with a fairly thick pile. Any veins which project below the leaf sink into this pile, and do not destroy the flatness of the upper surface, which therefore comes closely into contact with every part of the stamp.

The Ink.—Ordinary endorsing ink absorbs water from *Helianthus* leaves, and is therefore unsuitable. I have used "Ardinco Never-Smear" ink, which will stand a certain amount of rain, though it bleaches somewhat too readily.

Asymmetry tests by this method, made by taking pieces from both halves of the leaves at one and the same time, gave the following results:—†

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* It is advisable to select with care brass mounts with a perfectly plane surface, or to have them specially worked.

† The number of tests is small, as, owing to unfavourable conditions in the early part of the summer, the supply of suitable leaves was inadequate. All the best leaves were needed for assimilation experiments.

With the smallest stamp, differences per square decimetre of −8, +4, −14 and −10 milligrammes, or per cent. −1·9, +1·0, −3·4, and −2·2; with the 20 cm. stamp a difference of +16 milligrammes, or +3·2 per cent. (and another so great that I rejected it, as obviously due to mistake in weighing or in cutting out the area). The average difference per cent. was ±2·3. A comparison of this with the results obtained before by the templet method with *H. tuberosus** proves that the smallest stamp, measuring only 11·1 sq. cm., has given very satisfactory results.

Of the following experiments, the data are given in detail only for Expt. 2, which is interesting as an example of the use of small areas. In other cases, the *rate* of increase alone is given for each leaf; the pieces employed were usually 20, sometimes 40 sq. cm. in area, and in some cases a number of pieces were taken together, so that the total area from each half-leaf added up to 90 sq. cm. (Expt. 3).

Experiment 2.—July 20, 1909. Four small leaves, collected 6.45 A.M. Each half-leaf stamped at 8.50 A.M. Experimental half-leaves exposed at 9.15 A.M. Experiment closed at 4.15 P.M.; duration, 7 hours. Continuous bright sunshine, some haze. Maximum sun temperature, 27°·2 C.

Table II.—Area cut from each half-leaf, 11·1 sq. cm.† Asymmetry error per sq. decim. per hour: average about ±1 milligramme, maximum about ±2 milligrammes.

a = control half-leaf; *b* = experimental half-leaf.

Leaf.	Dry weight of 11·1 sq. cm., in grammes.	Gain of dry weight in 7¼ hours.	Gain per sq. decim. per hour, in milligrammes.	Condition of experimental half-leaf.
6	(*a*) 0·0340			
	(*b*) 0·0472	0·0132	17·0	Turgid.
7	(*a*) 0·0356			
	(*b*) 0·0457	0·0101	13·0	Turgid, then limp.
8	(*a*) 0·0300			
	(*b*) 0·0361	0·0061	7·9	Limp.
9	(*a*) 0·0306			
	(*b*) 0·0300	−0·0006	−0·8	Flaccid.

As in the first experiment, the rate of increase varied with the degree of turgidity, and leaves in similar states gave rates which agree closely in both experiments. The same agreement is to be observed in the other experiments. In Table VI (below, p. 430) the results are all tabulated for comparison.

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* See Thoday, *loc. cit.*, p. 16, Table IX.

† The pieces cut after stamping with the stamp reputed to be 10 sq. cm. are found to measure 11·08 sq. cm. on the average.

Experiment 3.—July 23, 1909. Five leaves gathered 5.30 A.M. Experimental halves exposed in the open air 8.30 A.M., removed to greenhouse at 10 A.M. owing to strong wind. Experiment closed 3.45 P.M.; duration, $7\frac{1}{4}$ hours. At first bright sun, clouded over gradually, but brightened again to fine sunny afternoon.

Table III.—Asymmetry error as in Expt. 2.

Leaf.	Rate of increase in milligrammes.*	Condition of experimental half-leaf.
10	17·1	Turgid.
11—14	5·3, 4·0, 4·3, 7·8	Flaccid to variable degrees.

* The rate of increase of dry weight is given throughout in milligrammes *per square decimetre per hour* unless otherwise stated.

Experiment 4.—August 6, 1909. Four leaves gathered 7 A.M. Experimental half-leaves set up under water-pressure of about $\frac{1}{2}$ metre, and exposed at 9 A.M. Water-pressure increased to $1\frac{1}{2}$ metre at 9.40 A.M. Set up canvas screen at 2 P.M. Experiment closed 5.10 P.M.; duration, $8\frac{1}{6}$ hours. Bright sunshine throughout; very little wind. Sun temperature, 27° to 29°·2 C.

Two pieces were cut from each half-leaf in the cases of Leaves 15 and 17, and were dealt with separately. Each pair of corresponding pieces from control and experimental half-leaves thus afforded a measurement of the rate of increase in the region of the leaf from which they were cut.

Table IV.—Asymmetry error: average about ±0·8 milligramme; maximum about ±1·6 milligramme.

Leaf.	Rate of increase in milligrammes.	Condition of experimental half-leaf.
15, tip	15·5	Fairly turgid, slightly limp about mid-day.
15, middle	14·1	
16	8·0	Slightly limp, occasionally more limp.
17, tip	8·5	Ditto.
17, base	4·1	Gradually more flaccid till drying.
18	−0·5	Flaccid throughout.

In this experiment none of the leaves remained perfectly turgid throughout the experiment, and none has increased in weight at so high a rate as the turgid leaves of Expts. 2 and 3.

It was observed that the part of the leaf towards the base and furthest from the midrib showed the greatest tendency to flag, while towards the tip the water supply appeared to be much more efficient. The results for Leaf 17 illustrate this.

Experiment 5.—August 7, 1909. Four leaves gathered 6.20 A.M., while still dewy. Experimental half-leaves exposed, under a pressure of 1½ metre of water, at 7.45 A.M. Canvas screen, transmitting 40 per cent. of the light, put up at 8 A.M. Experiment closed at 3.45 P.M.; duration, 8 hours. Bright sunshine throughout; some haze. Maximum sun temperature, 27°·8 C.

In the case of Leaves 19 and 22, two pieces were cut from each half-leaf and treated separately as in Expt. 4. The stamped areas were in all cases 19·8 sq. cm.

Table V.—Asymmetry errors as in Expt. 2.

Leaf.	Rate of increase in milligrammes.	Condition of experimental half-leaf.
19, tip	16·6	Turgid.
19, base	19·1	
20	18·6	
21	9·8	Stiffly turgid.
22, tip	9·7	Turgid, then limp.
22, base	10·5	

Leaf 22 was found to have flagged somewhat at 11 A.M., and from that time till the end of the experiment it remained a little limp, notwithstanding the great head of water under which it was set up. It showed a corresponding, rather low, rate of increase.

The other three leaves all remained throughout the experiment completely turgid. The high rates of increase shown by Leaves 19 and 20 are thus in accordance with expectation.

Leaf 21, on the other hand, showed a rate of increase comparable with that of Leaf 22. The result was surprising, as the leaf was observed during the experiment to be very stiffly erect, as if the head of water had freer play through its conducting channels than through those of any other leaf.

Observations which had been made during the experiment with a horn hygroscope of the form described by F. Darwin,* afforded no explanation of the result in question. Readings were taken at intervals of about an hour on both surfaces of each leaf, in the centre of each of the stamped areas. The average of the readings obtained on the upper and lower sides were as follows:—

Leaf 19, tip, $\frac{66}{77}$; base, $\frac{60}{71}$. Leaf 20, $\frac{57}{79}$. Leaf 21, $\frac{61}{72}$.
Leaf 22, tip, $\frac{57}{48}$; base, $\frac{34}{30}$.

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* "Observations on Stomata," 'Phil. Trans.,' 1898, B, vol. 190.

Thus the fully turgid leaves appear to have had their stomata open to approximately the same extent, Leaf 21 showing no difference from the others; whereas Leaf 22 had its stomata markedly less open.* The anomalous result with Leaf 21 may be due to an exceptional experimental error, or an unusually high degree of asymmetry; or it may be that the pressure of the excessively turgid epidermal cells upon the guard cells partially closed the stomata, and that increased evaporation under the head of water, which had freer play in this leaf than in the others, neutralised the effect of this closure upon the hygroscope.

Note on the Use of the Horn Hygroscope.—As the observations in connection with this and other experiments† had often to be made out of doors, it was necessary to protect the hygroscope from air currents, which greatly disturb its readings.

The protective mount was made of two parallel slips of glass, such as are used for microscopic preparations, attached to one another by lateral strips of card. The hygroscope just fitted into the space between the two glass slips and was affixed to them with shellac. This arrangement is shown in fig. 2. Readings were taken after about 10 seconds. After that interval a fall was observed, due presumably to the diffusion of water vapour into the partially enclosed air above the leaf, and the consequent decrease in the gradient of humidity.‡

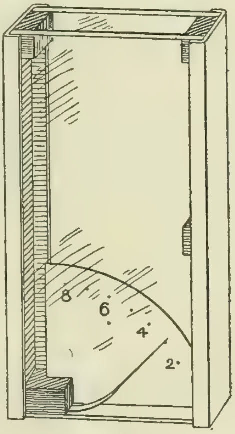

Fig. 2.

The conditions under which the hygroscope is used are variable in the open air, but it is probable that the readings obtained at the same time on different leaves are a true relative indication of the differing conditions of their stomata. Thus, in leaves which are losing their turgidity, the closing of the stomata is unmistakably revealed.

On the other hand, changes in the readings obtained with the same leaf from hour to hour are sometimes of doubtful significance, for the increasing internal temperature of the leaf, by directly increasing the rate of evaporation, must affect the hygroscope apart from any change in the stomata themselves. An effect of this nature is observed when readings are taken on a piece of wet blotting paper surmounted by fine wire gauze. If a series of observations be made first in the shade and then in the sun, the readings are found to increase as the temperature of the evaporating surface rises. The reverse effect is observed when the sunshine is intercepted.

In the sun, the occurrence of variations of leaf-temperature will make the hygroscope a less delicate indicator of the conditions of the stomata even in comparing different leaves at the same time; but, as a consequence of this loss of sensitiveness, any considerable relative change in the readings becomes still more significant.

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* The detailed observations show that in the case of this leaf the stomata, more especially those on the lower surface, began to close just after the leaf had begun to flag, at 11 A.M.

† I am indebted to Miss M. G. Sykes, Fellow of Newnham College, Cambridge, for many of the hygroscope observations taken in connection with the experiments. Without her kind assistance the series of observations would necessarily have been far from complete.

‡ The readings on the scale of the instrument were roughly degrees of arc with the point of attachment of the horn as centre.

(ii) *Discussion of the Results obtained with* H. annuus.

In the following table the results of all the experiments are collected together. The leaves have been separated into five classes, according to the degree of turgidity which they exhibited, and the results are so arranged that the rates of increase shown by leaves belonging to the same class in the different experiments may be compared. In the last column is given for each class the average rate of increase of all the leaves included in it.

Table VI.—All the figures are rates of increase of dry weight in milligrammes per sq. decimetre per hour.

Condition of leaves.	Exp. 1.	Exp. 2.	Exp. 3.	Exp. 4.	Exp. 5.	Average rate of increase.
(1) Turgid	17·2 13·8 16·3	17·0	17·1		16·6 19·1 18·6 (9·8*)	16·9 (16·1 including *)
(2) Moderately turgid, occasionally rather limp		13·0		15·5 14·1	9·7 10·5	12·5
(3) Limp........................	9·9	7·9		8·0 8·5		8·5
(4) Limp to flaccid............			5·3 4·0 4·3 7·8			5·3
(5) Quite flaccid from beginning of experiment	3·5	−0·8		4·1 −0·5		1·6

The agreement of these experiments among themselves is very satisfactory. The fact that the turgid leaves in Expt. 5 have given slightly higher results on the whole than those in Expt. 1, is, I think, a significant difference which is to be attributed to the different conditions under which the two experiments were conducted. Considering such differences in external conditions, and the range of variation possible in the condition of leaves in the same class, a small amount of variation beyond that which is due to asymmetry errors is to be expected in the individual results. The average rate of increase for the class is thus the mean of individual rates which vary within fairly narrow limits.

Sachs, who used large areas from seven leaves, obtained as the rate of increase in the dry weight of detached leaves in continuous sunshine

16·5 milligrammes per hour. *The very close agreement of this value with the average result for turgid leaves in the Table is conclusive.**

Such a high rate of assimilation has always been regarded as remarkable. Now that it has been confirmed its significance is profound.

For instance, such rapid assimilation is only possible with a *high leaf-temperature.* Blackman and Matthaei have shown† that for any given temperature there is a maximum rate of assimilation which cannot be exceeded unless the temperature is raised. If a moderate correction be made for the assimilation of the carbon dioxide formed in respiration,‡ the total assimilation becomes equivalent to a gain of about 18 milligrammes of dry substance per hour. Assuming that Blackman and Matthaei's temperature assimilation curve for *Helianthus tuberosus*§ holds approximately also for *H. annuus*,‖ it follows that the minimum temperature is between 23° and 24° C.

The temperature recorded by a bright mercury thermometer in the open during the experiments was usually above this temperature; and all the available evidence¶ points to the conclusion that the internal temperature of the leaves themselves is several degrees higher still. Where the thermometer recorded temperatures as low as 20° (after the canvas screen was first put up, in Expt. 5), or 21–22° (as in the cloudy intervals during Expt. 3), the leaf temperature was probably slightly higher than this; although, considering the exceptionally rapid transpiration of this leaf, it is questionable whether it reached the minimal temperature. Thus at times the rate of assimilation may have been less than the average: if so, it must also at times have been more than the average.

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* The fact that at the end of his experiment Sachs had to reject one half-leaf because it had wilted, and thought it advisable to float the others on water, led me to infer that even these had become somewhat limp; but from the result which he obtained it seems a necessary inference that the leaves remained turgid till very near the end of the experiment, and that Sachs stopped the experiment as soon as they began to droop.

The result obtained by Brown and Morris, 10·0 milligrammes, is intermediate between those given by leaves in the second and third categories in the table, *i.e.*, it corresponds to a somewhat limp condition.

† Matthaei, 'Phil. Trans.,' 1904, B, vol. 197; Blackman, F. F., 'Annals of Botany,' 1905, vol. 19; also Blackman and Matthaei, 'Roy. Soc. Proc.,' 1905, B, vol. 76.

‡ See Brown and Escombe, 'Roy. Soc. Proc.,' 1905, B, vol. 76, p. 69. The starch equivalents of the values there given for the respiration of leaves of *H. annuus* are, at 19°·6 C., 0·7 milligramme per hour; at 31°·2 C., 20 milligrammes per hour. As in my experiments the leaf-temperature was probably never lower than 20°, usually nearer 30°, and may well have exceeded 30° at times, 1·5 milligramme is a medium estimate of the average rate of respiration.

§ See *loc. cit.*, 1905, p. 413, and fig. 2 on p. 414. Blackman and Matthaei there record a rate of assimilation at 22°·3 of 13·1 milligrammes CO_2 per 50 sq. cm. per hour, which is equivalent to 16·1 milligrammes *starch* per *sq. decimetre.* (On the use of the starch equivalent in calculating the increase of dry weight corresponding to a given intake of CO_2, see Thoday, *loc. cit.*, p. 10.)

‖ Considering the similar character of the leaves of *Helianthus annuus* and *H. tuberosus* it would seem to be a justifiable assumption that, when the supply of carbon dioxide and radiant energy are abundant, their rates of assimilation at any given temperature will be approximately identical. *Cf.* the curves given by Blackman and Matthaei for such different leaves as *H. tuberosus* and Cherry Laurel, *loc. cit.*, p. 414.

¶ Direct experimental evidence of such differences of temperature between leaves and their surroundings, especially when exposed to sunlight, has been given by Blackman

Still Higher Rate of Assimilation Possible.—This suggestion is made probable by observations which were made with the horn hygroscope. These show considerable variations during an experiment lasting several hours. On the whole, the readings increase gradually towards noon, reaching a maximum about that time. They then remain more or less constant for one or two hours, and afterwards gradually fall.

Although the exact interpretation of the individual readings is open to doubt, yet as a whole they clearly support the general conclusion that the condition of the stomata is not uniform over so many hours. If, as we have assumed to be generally the case, the factor which limits assimilation is, throughout, the supply of carbon dioxide, it follows that the rate of assimilation must vary during the experiment.

The rate of increase may, then, be even higher than 18 milligrammes per hour. The maximum rate cannot be accurately estimated, but we may safely conclude that it is above 20 milligrammes.

This being so, a minimal temperature as high as 25° C. may sometimes be required for the leaf to make full use of the supply of carbon dioxide which its widely opened stomata make possible.

The need for such high temperatures suggests that, even in ordinary air, temperature may be more often than has been suspected the factor limiting assimilation. Such a state of things might occur, for instance, on a bright cold day, unless the low temperature prevent the stomata from opening to their fullest extent notwithstanding bright illumination, and thus limit the rate of assimilation indirectly.

The Waste of Photosynthetic Radiation.—Blackman and Matthaei* have calculated the proportion of the energy of sunlight, available for assimilation, which is actually used by leaves under various external conditions, natural and experimental.

For *Helianthus annuus* they estimated the waste of energy in ordinary air

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and Matthaei (*loc. cit.*, 1905) and by A. M. Smith ("On the Internal Temperature of Leaves in Tropical Insolation," 'Ann. Roy. Bot. Gard., Peradeniya,' 1909, vol. 4).

* *Loc. cit.*, p. 455.

from the rate of increase found by Brown and Morris (10 milligrammes per hour per square decimetre), as it appeared at the time to be a safer value than that of Sachs.

It now becomes of interest to recalculate this waste from the much higher rate of increase which has now been established.

Blackman and Matthaei estimated the total radiation available on a representative sunny day as sufficient for the assimilation of 53 milligrammes of CO_2 per hour per 50 sq. cm. of leaf surface. This is equivalent to the formation of 65 *milligrammes of starch per square decimetre per hour.* An increase of dry weight of 18 milligrammes per hour is about 30 per cent. of this possible 65 milligrammes, and *there is thus a waste of about* 70 *per cent. of the energy which could be utilised*; whereas Brown and Morris' figure represents a waste of over 80 per cent. Since there is some evidence for still higher rates of assimilation the waste may be at times reduced to 65 per cent. or less.

(iii) *Brown and Escombe's Experiments on Leaves of* H. annuus.*

The results which Brown and Escombe obtained with leaves of *H. annuus* enclosed in their experimental case are almost of a lower order of magnitude than those described here. The highest rate of assimilation which they observed corresponds to an increase in dry weight of 5·5 milligrammes per square decimetre per hour. This must be regarded as demonstrating that the conditions holding in their experiments were widely different from those occurring in the open air.

In the first place they found it impossible to carry out experiments successfully in full sunshine as the temperature was injuriously high. The resultant gaseous exchanges were reduced practically to *nil*, owing partly to the increased intensity of respiration, partly perhaps to the reduction of assimilation by the injurious action of the high temperature.

On the other hand, in their experiments in moderated illumination Brown and Escombe considered that the leaves were at no disadvantage compared with leaves under natural conditions; for they found that the intensity of the illumination under their canvas screen in sunlight was greatly in excess of the capacity of leaves of *Tropæolum majus* for assimilation in ordinary air.

That they were justified in generalising, from experiments with a single species, as to the sufficiency of the supply of energy for the rates of assimilation with which they were dealing, follows from Blackman and Matthaei's demonstration that experiments with leaves as different as those of Cherry

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* *Loc. cit.*, Table I.

Laurel and *Helianthus tuberosus* give concordant results for the photosynthetic value of a given intensity of light. In other words, " the same amount of CO_2 is reduced in both plants by the same intensity of light."*

Brown and Escombe's screen, then, permitted in full sunlight much more light to pass than was required for the highest rate of assimilation which they observed,† and their low values are not to be explained as due to the limiting of assimilation directly by the supply of energy.

Light, however, influences the rate of assimilation in nature indirectly through the stomata, and in this respect to reduce the intensity of the illumination is to run the risk of putting the leaf at a disadvantage.

Darwin‡ has shown how sensitive stomata are to changes in the intensity of the light. In one experiment he shaded a leaf of *Cucurbita*, growing in bright diffused light, with thick netting, and found that in less than an hour the horn hygroscope gave a reading only one-half of that which it had given before, while on an unshaded control leaf the reading remained practically constant. He also observed that on a dark wet day in August as well as on dark winter days many leaves gave zero readings with the horn hygroscope.

I have made one assimilation experiment with *H. annuus* on a dull rainy day in August, 1909. The few hygroscope observations which could be made during the experiment were in agreement with these results which Darwin obtained with greenhouse plants, the readings being low, though not zero. The values obtained for the rate of assimilation were also very low, lying between 1 and 2 milligrammes; but exposure to rain had made the stamped rectangles very faint and the results are therefore not fully trustworthy.

Although this evidence deals with light of low intensity, the fact that the width of the stomatal openings and the rate of assimilation are very small suggests that some direct relation to the intensity of light may exist through a considerable range of intensity. Assuming that such is the explanation of Brown and Escombe's low results it follows that even bright diffuse light, to which their leaves were exposed, is not sufficient to bring about full opening of the stomata.

Relation of the Stomata to Illumination.—What are the conditions necessary to induce the stomata to open widely is a question which cannot fully be answered, but there are some further facts which may bear on it.

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* Blackman and Matthaei, *loc. cit.*, p. 445.

† Calculating from their Expt. 3 with *Tropæolum majus*, after introducing an appropriate correction for respiration (from Blackman and Matthaei, *loc. cit.*, p. 446), the full illumination possible under this screen was sufficient for the formation of 10 milligrammes dry substance per hour. Their highest rate of assimilation was equivalent to about 5·5 milligrammes.

‡ *Loc. cit.*, pp. 573—4.

In my Expt. 5 the leaves were shaded from the full force of the sun's rays by a screen of coarse canvas which allowed only 40 per cent. of the light to pass. The high rate of assimilation was nevertheless maintained, showing that the stomata were fully open. The photosynthetic value of diffuse light may often be approached, sometimes even exceeded, by that of the light which passes through this screen in full sunshine. For instance, Blackman and Matthaei, in discussing the waste of available energy in nature, take as an example of moderate intensities of illumination on a sunny day a case in which the diffuse light alone allowed of 32 per cent. as much assimilation as the whole of the available radiation. Yet Brown and Escombe, exposing the leaf to a diffuse light from a northern sky, observed a rate of assimilation less than one-third of that which was given by the turgid leaves under my canvas screen. Assuming that Brown and Escombe's leaves were also fully turgid, it seems to follow that some quality of the light other than its photosynthetic value induced the stomata to open more widely in my experiment. This factor may possibly be the greater preponderance of heat rays in the direct sunshine, and the consequent raising of the leaf-temperature.

Brown and Morris' experiment on a dull day gave as the rate of increase of dry weight 9·8 milligrammes. This is a little more than half the rate reached in full sunlight. If the result is not excessive owing to shrinkage errors the diffuse light must have been bright compared with that available during my experiment on a dull day, mentioned above, notwithstanding the fact that the very low result in the latter case is partly to be explained by the blocking of the upper stomata by the rain. Therefore, also, in this experiment of Brown and Morris, the photosynthetic value of the illumination may have been nearly equal to that under my canvas screen;* if so, some other quality of the radiation must again account for the great difference in the rate of assimilation.

The possibility of leaf-temperature and intense illumination affecting the stomata has received little attention hitherto. Whether the suggestion here made can be substantiated remains to be seen, but one thing seems clear, that the stomata of *H. annuus* do not open fully in even bright diffuse light.

Section III.—EXPERIMENTS WITH *Catalpa bignonioides.*

Helianthus annuus is probably exceptional, even among herbaceous plants, in its high rate of assimilation. Other rapidly growing plants, such as

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* *Cf.* high maximum rate of assimilation during *dull* spell, Blackman and Matthaei, *loc. cit.*, p. 442, Expt. 16.

Table VII.

Leaf.	Rate of increase, in milligrammes.	Average of hygroscope readings.	Experimental data.
			Experiment 6.
1	8·4	31	August 4, 1909 : *detached leaves.* Cut, 7.30 A.M. Exposed in open from 11 A.M. till 5.15 P.M. (6¼ hours). Maximum sun temperature, 27°·8 C. Bright sunshine, with rare clouds ; breezy. Stamping method : Leaves 1—7, 40 sq. cm.; asymmetry error, average ±2·0 milligrammes, maximum ±4·5 milligrammes. Leaves 8—12, 20 sq. cm. ; asymmetry error, average ±3·5 milligrammes, maximum ±7 milligrammes.
2	0·4	31	
3	2·4	35	
4	0·6	25	
5	3·0	33	
6	8·3	36	
7	10·2	33	
8	3·7	35	
9	3·8	38	
10	2·5	38	
11	12·9	40	
12	7·2	26	
Average	5·3	33	Probable asymmetry error ±0·7 milligramme.
			Experiment 7.
13	6·4	69*	July 6, 1908 : *detached leaves.* Cut, 7 A.M. Exposed in greenhouse from 10.20 A.M. till 4.30 P.M. (6 hrs. 10 mins.). Cloudy, clearing to bright sunshine. Maximum sun temperature, 32° C. Templet method : 40 or 50 sq. cm. Correction for shrinkage by marking leaves.† Asymmetry error, average ±2·5 milligrammes, maximum ±4 milligrammes.
14	8·6	54	
15	−0·7	51	
16	4·6	44	
17	3·7	39	
18	−1·5	23	
Average	3·5	47	Probable asymmetry error ±1 milligramme.
			Experiment 8.
19	5·4	44*	July 3, 1908 : *attached leaves.* 7 A.M. to 3 P.M. (8 hours). Shade temperature, 22—23° C. Continuous sunshine. Templet method : 40 or 50 sq. cm. Correction for shrinkage by marking leaves.† Asymmetry error, average ±2 milligrammes, maximum ±3 milligrammes.
20	2·6	36	
21	−0·4	36	
22	6·7	32	
23	2·8	29	
Average	3·4	35	Probable asymmetry error ±1 milligramme.

* Different hygroscopes were used in the three experiments, so that the readings are only comparable within a single experiment.

† See p. 423 and Thoday, *loc. cit.*, pp. 23 and 37.

Cucurbita,* may prove to be close rivals of *Helianthus*, and Weber's results† suggest that *Ricinus communis* and *Tropæolum majus* are not far behind.

Catalpa, on the other hand, is a tree, and although like *Helianthus* it is comparatively large-leaved and grows fairly rapidly, its leaves have stomata only on their lower surface, and therefore cannot be expected to show such high rates of assimilation. It is not surprising, therefore, that in the following experiments the highest rate of assimilation is far below corresponding rates for *Helianthus* obtained in the experiments described in Section II.

The results given by individual leaves of *Catalpa* in the same experiment do not show the same agreement as was found with *Helianthus*; instead they vary within very wide limits, even when, as in Expt. 6, the apparent condition of the different leaves was remarkably uniform. This is chiefly due to the high degree of asymmetry in respect of dry weight per unit area shown by the leaves of this plant. The following asymmetry tests by the stamping method form the basis of the asymmetry error estimated for Expt. 6, in which that method was used:—

Area taken, 11·1 sq. cm.‡

Differences found, milligrammes per sq. dec.... +21, +34, +26; average, ±27.
Per cent. .... +5·1, +8, +6·7; average, ±6·4.

Area, 19·8 sq. cm.

Differences found, milligrammes per sq. dec.... −18, −43, +3, +33, +14, −13; average, ±21.
Per cent. .... −4·3, −11·2, +0·7, +5·7, +2·2, −3·1; average, ±4·5.

Area, 38·1 sq. cm.

Differences found, milligrammes per sq. dec.... +25·6, +8·3, −1·7, +21·6, −2·5; average, ±11·8.
Per cent. .... +4·6, +1, −0·4, +4·4, −0·5; average, ±2·2.

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* *Cf.* Sachs' results for attached leaves of *Cucurbita* and *Helianthus*, *loc. cit.*

† "Ueber specifische Assimilationsenergie," 'Arb. d. Bot. Inst. Würzburg,' vol. 2, p. 346. See Sachs, 'Lectures on the Physiology of Plants,' 1887, p. 313.

‡ These results are, on the whole, comparable with those previously obtained by the templet method (Thoday, *loc. cit.*, p. 18; *cf.* also Brown and Escombe, *loc. cit.*, p. 60). One point of importance arises from them. The results obtained from the 10 sq. cm. stamp between the main ribs show greater disparity between the weights of corresponding pieces from the two halves of leaves than do the results with larger pieces in which ribs were included. This I attribute to the irregular distribution of minor outstanding veins, which cannot be avoided even in selecting very small areas. It also appears that pieces 40 sq. cm. in area give better results than 20 sq. cm. pieces. In assimilation experiments with *Catalpa* the smallest stamp was discarded, and the largest was used wherever possible.

These differences have, of course, to be divided by the number of hours of insolation to find their effect upon the observed rate of increase of dry weight.

The corresponding data for Expts. 7 and 8 were calculated from the results of asymmetry tests by the templet method given in the earlier paper.*

It will be observed that the maximum asymmetry error would account for practically the whole range of variation among the individual results. For instance, in Expt. 6, Leaves 8 to 12, the maximum error of 7 milligrammes on either side of the average rate would give −1·7 milligrammes and +12·3 milligrammes. The considerable variations, however, in the hygroscope readings on the different leaves indicate that the true rate of assimilation itself varied from leaf to leaf, so that part of the variation in the results is to be accounted for in this way.

This is true, even in Expt. 6, where almost all the leaves preserved a uniformly turgid appearance throughout the time during which the experiment lasted.†

Catalpa, however, is unlike *Helianthus*, in that it is not possible to gauge accurately the condition of the stomata, nor even the turgidity of a leaf, from its external appearance; for loss of turgidity is not at first accompanied by readily visible symptoms. The hygroscope readings which were made at intervals on each leaf are therefore of the greater value.

A comparison between Expts. 6 and 7 shows that when signs of flaccidity do appear the effect on the assimilation is marked. In Expt. 6, corresponding with the uniformly turgid appearance of the leaves, the average rate of assimilation was 5·3 ± 0·7 milligrammes per hour; whereas in Expt. 7, in which several of the leaves became flaccid, the much lower average rate, 3·5 ± 1·0 milligrammes, was found.

Taking into account the variations indicated by the hygroscope readings, greater rates of increase than 5·3 milligrammes per hour are to be expected in fully turgid leaves. The rate of increase for Leaf 11, with an average hygroscope reading of 40, should be about 6·4 ± 0·9 milligrammes per hour.

These figures may be compared with Brown and Escombe's highest value, obtained during four hours of intermittent sunshine. Measuring directly the amount of carbon dioxide absorbed by a detached leaf enclosed in their experimental case, they obtained a rate of assimilation approximately equivalent to an increase of dry weight of 4·7 milligrammes per hour. My results, by the dry weight method, are in fair agreement with this gasometric result,* but indicate more rapid assimilation in continuous sunshine; and it may be true for *Catalpa*, as well as for *Helianthus*, that before the highest rate of assimilation can be attained the leaves must be exposed to conditions approaching full insolation.

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* *Loc. cit.*, p. 18.

† The only noteworthy exception was Leaf 12, which flagged visibly in the afternoon, and for which the average hygroscope reading is correspondingly low. The low average reading for Leaf 4 is due to its having postponed the opening of its stomata till later than the other leaves.

Expt. 7 exhibits within itself the effect of the closure of the stomata in diminishing the rate of assimilation. In spite of the great irregularities due to asymmetry errors, a general gradation is visible in the rates of increase following the variation in the averages of the hygroscope observations. The highest rates of increase are shown in the case of leaves with their stomata most widely open.

Calculating from the average result, on the assumption that the hygroscope readings are proportional to the individual rates of assimilation, a closer approximation to the rate of increase for Leaf 13, with its stomata most widely open, is $5·1 \pm 1·5$ milligrammes. This is close to the average rate in Expt. 6. It is to be noted that in Expt. 7 the sky was at first cloudy; in continuous sunshine yet a higher value would probably have been obtained.

In Expt. 8 the leaves were still on the tree, and the average rate of increase is much lower than in Expt. 6, although the leaves remained in appearance turgid, and the sunshine was continuous throughout the day. This difference is, in part, to be explained by less widely open stomata, for Brown and Escombe's simultaneous experiments with attached and detached leaves, and observations of my own with the horn hygroscope, both show that detached leaves tend to open their stomata more widely than leaves still attached to the tree. This point will be discussed more fully in the next section.

Section IV.—Remarks on the Rate of Assimilation in Nature; and on the Occurrence of Translocation During the Day.

In the previous sections it has been shown that very high rates of assimilation are possible to leaves of *Helianthus annuus* when detached from the plant, and that leaves of *Catalpa* also may under like conditions assimilate more rapidly than has hitherto been observed. It therefore becomes of importance to inquire how far the conditions necessary to these high rates are actually realised in nature, *i.e., in leaves which are still upon the plant.*

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* Brown and Escombe's results for *Catalpa* by the dry weight method do not agree with their gasometric results nor with my dry weight results, but vary up to 16·6 milligrammes per hour (average for four leaves). This is due, as has already been pointed out (*loc. cit.*, p. 52), chiefly to error from shrinkage in area, to the possibility of which Brown and Escombe were the first to draw attention.

It was assumed by Sachs that leaves were in the same condition, in so far as their rate of assimilation was affected, whether on the plant or detached from it. Thus he regarded the fact that attached leaves of *H. annuus* showed a much smaller rate of increase of dry weight than detached leaves as due entirely to the translocation of part of the products of photosynthesis from the attached leaves into other parts of the plant. In support of this view he adduces, from his own results, the concordance of the rate of increase of detached leaves, 16·5 milligrammes per square decimetre per hour, with the value obtained when the rate of translocation from attached leaves at night, 9·6 milligrammes, is added to their rate of increase during the day, 9·1 milligrammes.

Brown and Escombe* have, however, pointed out that it is not justifiable in the absence of direct experimental evidence to assume that leaves on the plant fix as much carbon dioxide as detached leaves. Even under identical external conditions of temperature, illumination, humidity, and carbon dioxide content of the air, the condition of the stomata may be different after detachment.

With the object of finding whether such differences of stomatal aperture actually occur they made measurements of the amounts of carbon dioxide diffusing into the leaf during the same time and under the same conditions in two similar leaves of *Catalpa*, one still on the tree, the other detached. Two such experiments both showed a considerable excess in favour of the detached leaf. The inference is that the stomata of the latter were more widely opened.

It is clear that these results establish their contention that the possibility of such differences must be considered in comparing the results of experiments with attached and detached leaves; but they went further than this: they pointed out that, as they believed, the rate of increase of attached leaves of *H. annuus* bears a relation to the rate of increase of detached leaves comparable with that which they found in *Catalpa*. Hence they maintained that in the case of *Helianthus* also the difference is to be attributed to the wider opening of the stomata of detached leaves, and denied altogether Sachs' assumption that translocation and assimilation proceed concurrently.

On the other hand, it must be remembered that, although not fully justified by the particular results to which he applied it, Sachs' assumption was not purely gratuitous, but was founded upon numerous observations made upon leaves at different hours of the day under varying external conditions by the iodine starch test.

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* *Loc. cit.*, p. 51.

Moreover, Brown and Morris' dry weight experiments with *Helianthus* support Sachs rather than Brown and Escombe. That they should have appeared to harmonise so well with the *Catalpa* experiments quoted above was due to a misquotation of the result of one of Brown and Morris' experiments, in which the attached leaf is quoted as showing an increase of 4·6 milligrammes per square decimetre per hour,* but according to the original only increased by 4·6 milligrammes in *nine* hours, an amount so small that it was neglected as coming within the errors of experiment.†

Thus while the detached leaves increased in weight at the rate of 10 milligrammes per hour, the attached leaves showed no increase at all. There is no reason whatever for supposing that the stomata of all the eight attached leaves were completely closed during the whole period of 12 hours covered by the experiment.‡ It therefore remains impossible to doubt that a considerable quantity of substances was translocated from the leaves during that period. In no other way can we adequately account for the lack of accumulation of the products of photosynthesis.

The relation between the stomatal apertures of the attached and detached leaves in this experiment of Brown and Morris is another question, and one which cannot be decided in the absence of the necessary data. It may not be unprofitable, nevertheless, to consider how far the results of Brown and Escombe's experiments with *Catalpa* are likely to be applicable to other leaves. To do this it is necessary to inquire into the conditions which produced the differences of stomatal aperture which they found.

The Effect of Detachment from the Plant upon the Supply of Water to Leaves.—The difference in the osmotic quality of the water supply has been suggested as a possible factor; attached leaves receive water charged with mineral salts from the soil, whereas detached leaves are supplied with distilled water.§ It seems, however, unlikely that the concentration of mineral salts in the sap would ever be great enough to produce an appreciable diminution of turgor in the leaf, or to induce any movement on the part of the stomata.

Of far greater significance is the change, accompanying detachment, in the mechanical conditions which govern the water supply. During vigorous transpiration a considerable negative pressure has been shown to exist in the water channels of the stem, and this means, necessarily that considerable resistance must be overcome by the leaf in absorbing water from the conducting tracts immediately below its petiole.

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* Brown and Escombe, *loc. cit.*, p. 50, Table IV.

† Brown and Morris, *loc. cit.*, pp. 626—7.

‡ The day was "dull": it is therefore unlikely that transpiration was rapid, and so any large difference of stomatal aperture between attached and detached leave cannot reasonably be assumed.

§ Brown and Escombe, *loc. cit.*

When, on the other hand, the leaf is cut from the stem and the cut end of its petiole immersed in water, that portion of the resistance which was due to tension disappears altogether. The absorption of water therefore becomes easy, so long as other sources of resistance do not come into play.

In actual experience it is difficult to avoid for long the blocking of the vessels. Even when the petiole is cut through while immersed in water, air is apt to enter the vessels from the air spaces. When this does not occur the vessels sooner or later become clogged with mucilaginous substances from the cut cells. When ordinary water containing air is supplied, air-bubbles are liberated in the water channels and block them completely, especially when the leaf is exposed to the sun.

It appears, then, that (1) initially, under the most favourable circumstances, detached leaves experience relatively little resistance to the absorption of water, and are therefore likely to open their stomata more widely than leaves still on the plant, where the water supply is obtained with greater difficulty; but that (2) sooner or later, owing to the blocking of their water channels, they are likely to close their stomata, and so come to be at a disadvantage compared with attached leaves.

Brown and Escombe's experiments with *Catalpa*, already quoted, illustrate the initial phenomenon, and I have obtained confirmatory evidence with leaves of the same plant by means of the horn hygroscope. Leaves which had been detached since 7 A.M., and exposed to sunlight since 10.20 A.M., gave readings at noon ranging between 67 and 58, whereas attached leaves gave readings between 49 and 53. In the afternoon, however, several of the same detached leaves gave very low readings, having begun to lose turgor, thus illustrating the second phenomenon.

The relative duration and importance of these two relations must vary with the nature of the plant and the conditions of experiment. The initial opening of the stomata will be most marked in the case of trees and in bright sunshine; it will be less marked or absent on moist dull days, and, with many herbaceous plants, the water supply to leaves on the plant may be abundant enough to allow the stomata, even in sunshine, to open to their widest extent.* Some observations which I have made indicate that this is true for *Helianthus annuus*.

Two leaves were detached about 9.30 A.M. on September 18, 1909, and a portion of the stalk of each was at once removed under air-free water. Parallel readings with the hygroscope made on these and on two similar attached leaves at intervals during several hours of intermittent sunshine showed no differences that could be attributed to detachment.

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* When root pressure reaches as far as the leaves, detachment would reduce the efficiency of the water supply, and might be followed by some reduction of the stomatal apertures. Such phenomena are especially probable in low herbaceous plants.

Also, observations made at the beginning of September, 1909, on attached leaves gave readings nearly approaching those obtained with the same hygroscope on the attached leaves of Expt. 5, though the conditions were less favourable.

These observations do not support the assumption that attached leaves of *H. annuus* are at a disadvantage compared with detached leaves. I conclude, therefore, that so long as attached leaves of this plant present a fully turgid appearance, they assimilate at an equal rate with turgid detached leaves.

The Occurrence of Translocation.—On these grounds, and from a study of the details of Sachs' experiments with *H. annuus*, and those of Brown and Morris, it seems to me certain that the differences which have been found between the rates of increase of attached and detached leaves under the same conditions of illumination are due not to any great extent to differences in the stomatal apertures of the leaves, but chiefly to translocation; and that the rate of translocation may approach the value which Sachs assigned to it.

This conclusion cannot be extended to other leaves without experimental evidence. In fact, my Expts. 7 and 8 with *Catalpa* tell rather against than for the occurrence of translocation there. Even for *H. annuus* it must be remembered that the conclusion deals with average results obtained from experiments lasting a number of hours, and leaves quite open the question whether translocation is practically continuous or whether it only begins after a considerable quantity of reserves has been stored up.*

Broocks' Experiments with the Sugar Beet.†

In connection with these questions of stomatal aperture and translocation, some remarkable results obtained by Broocks are well worth attention. By means of the dry-weight method he carried out some very laborious experiments on leaves of the sugar beet, with the object of investigating the rate of assimilation from hour to hour during the day. He took the control half-leaves at 6 A.M. and the experimental halves in lots of 15 leaves each at 9 A.M. and every succeeding hour till 6 P.M. The correspondence between the results which he obtained on two continuously sunny days is so striking that they deserve consideration, notwithstanding the large errors from shrinkage in area which may be included in them. As Broocks' paper, so far as I am aware, has never been published in any scientific journal and is not readily available, I reproduce his results here.

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* Müller describes an experiment with attached leaves of *H. annuus*, the results of which show a uniform increase in weight at the rate of about 10 milligrammes per hour, continuing from 7.30 A.M. to 5.30 P.M. ('Jahrb. f. wiss. Bot.,' 1904, vol. 40, p. 468, Expt. 30). This suggests that translocation is continuous in the case of this leaf, and proceeds concurrently with assimilation.

† Broocks, W., "Über tägliche und stündliche Assimilation einiger Kulturpflanzen," 'Inaug.-Diss.,' Halle, 1892.

In fig. 3 the results of both the experiments in question are plotted; the ordinates represent the total accumulation of dry substance since the beginning of the experiment.

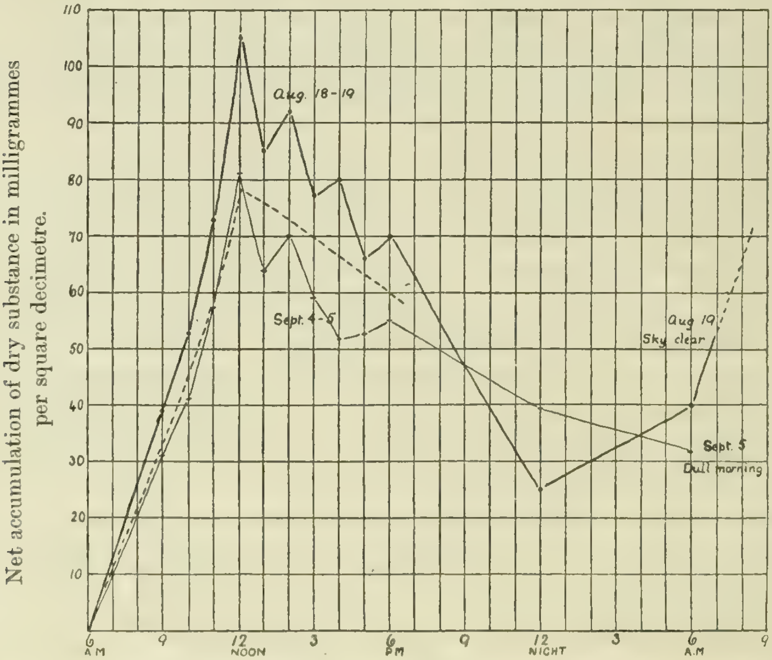

FIG. 3.—Curves showing variation in relative amount of products of photosynthesis present in leaves of sugar beet at different hours of two continuously sunny days and the nights following them. Constructed from Broocks' data. Ordinates represent excess of dry weight per square decimetre over that at 6 A.M. when experiments were begun. For explanation of broken line see text.

Broocks followed up each of these two experiments by measurements of the loss of dry weight between 6 P.M. and midnight, and between 6 P.M. and 6 A.M. the next morning. His results are added as continuations of the same curve.

The broken line represents the results for the first experiment, that of August 18, after making a deduction for the *apparent* change of dry weight which would have been observed, apart from true photosynthetic increase, if the leaves had changed in area as much as leaves of *Helianthus annuus* have been observed to do.* This correction for shrinkage would be extreme, so far as my observations go, as I have not found that leaves of the sugar beet shrink so much as those of *Helianthus*.† Even after this large correction for shrinkage the change in the curve is but slight, either in form or in the magnitude of the quantities it represents.‡

The chief peculiarity common to both Broocks' curves strikes the attention at once. Until noon they indicate a rapid increase of dry weight, but at noon their direction is suddenly reversed, and from that time they represent instead a decrease.

Another interesting feature is the continuity on both days between the curves of diminishing weight in the afternoon and from 6 P.M. to midnight. The obvious inference is that the same interpretation holds for the entire period of 12 hours and we may conclude that translocation took place at a nearly uniform rate throughout the period in question.

The change in the direction of the curve at noon shows that assimilation practically ceased at that time; a conclusion which also follows from the interpretation just given of the continuity of the latter part of the curve. It appears probable that this cessation was due to the closing of the stomata, for no other explanation would account for its suddenness.

This closure might have been brought about by loss of turgor in full insolation. It appears more probable, however, that it followed upon repletion of the leaf tissues with the products of photosynthesis; for it will be observed that in neither experiment had the leaves reached their original weight by the next morning, so that by the time they checked their assimilation they had already formed more products than could be translocated before assimilation recommended.

An interesting difference is to be noticed between the falling portions of the two curves. In the experiment of August 18–19, the translocation was rapid and uniform till midnight, but by 6 A.M. the weight had increased once more: the morning was bright, the sky clear, and assimilation had begun.

The morning of September 5, on the other hand, was dull and cold, and the curve shows no sign of rising. It is also to be observed that in this experiment translocation is less rapid than it had been three weeks earlier, and its rate gradually decreases. This decrease may be due to the fall in temperature which took place during the night. As a whole, however, the lower rate cannot be explained in this way, as the temperature was rather higher than on August 18–19. It is probably to be correlated with the slower growth of the tuberous roots so late in the season or their approaching repletion with stored reserves.

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* Thoday, *loc. cit.*, p. 24.

† *Cf. ibid.*, p. 34.

‡ Similar results are to be found among those described by Müller in 'Jahrb. f. wiss. Bot.,' 1904, vol. 40. See Expt. 10, p. 454, *Rumex*; Expt. 14, p. 456, *Rumex*; and Expt. 21, p. 462, *Verbascum*.

There remains for consideration the question whether translocation began after the closure of the stomata, or whether it was practically continuous so so long as reserves were present to be translocated.

There seems to be little reason for supposing any connection to exist between the closure of stomata, or cessation of assimilation, and the initiation of translocation.

The only evidence which might be taken to bear against the view that translocation takes place during assimilation has reference to the formation of diastase in leaves. Brown and Morris* have found that diastase is formed in increasing amounts as translocation proceeds in the dark, and they have been able to find no signs of its activity in the starch of actively assimilating leaves. They suggest, therefore, that no dissolution of starch takes place while starch formation is in progress. This view is, however, quite consistent with the supposition that translocation of a part of the soluble sugars, which are formed in photosynthesis, takes place at the same time as another part is condensed into starch. If, as they have suggested, the secretion of diastase is to be regarded as a sort of "starvation phenomenon" which sets in only when the quantity of soluble sugar is diminished, the formation of a superabundance of sugars during assimilation would be sufficient to inhibit diastase secretion, even though translocation were proceeding at the same time. Indeed, in expressing their views on the periodicity of diastase secretion, Brown and Morris seem to imply the continuity of the translocation of soluble sugars.

Moreover, it must not be forgotten that the products of photosynthesis are not translocated only in the form of sugars, but that proteids also are formed in the leaves.

The high rate of assimilation in Broocks' experiments tells at first sight against concurrent translocation. Broocks' results for August 18 indicate, as they stand, an average rate of increase between 6 A.M. and noon of 17 milligrammes per hour per square decimetre. Introducing a moderate correction for shrinkage, the value remains still as high as about 14 milligrammes. If we assume that translocation proceeded during this same period at the same rate as during the latter part of the experiment, another 3 or 4 milligrammes must be added, making the total rate 17 or 18 milligrammes.

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* *Loc. cit.*

In the light, however, of the high rate of increase shown by detached leaves of *Helianthus annuus* this value for the sugar beet is by no means incredible, especially when we consider that the air near the ground is more highly charged with carbon dioxide than is the normal atmosphere; no doubt is therefore thrown upon the assumption of translocation which was made in obtaining this value.

Other evidence.—The same assumption also makes possible the interpretation of some of Müller's results.* Several of his comparative experiments with Monocotyledons and Dicotyledons appear to indicate that leaves of the former class increase in weight much more rapidly to begin with than leaves of Dicotyledons, continue assimilating at their full capacity for a shorter time, and translocate the products much more slowly.

It appears possible that both the greater rapidity and the shorter duration of the initial increase in Monocotyledons are to be explained by reference to the slow translocation. If it be assumed that translocation continues side by side with assimilation, the more rapid translocation in Dicotyledons would reduce the net increase in weight more than the slow translocation in Monocotyledons; and in the latter, *ceteris paribus*, repletion would be reached more quickly.†

This slower translocation in Monocotyledons is probably connected with characteristic structural and biological features, such as the relatively small and slowly growing stem, and the long parallel-veined leaves. It would indeed be strange were the rate of translocation found to be independent of such features. The whole subject of translocation is one which promises a rich and varied field for research, and calls for the united efforts of physiologist and physiological anatomist in its investigation.

Section V.—SUMMARY.

1. In this paper it is proved that in the open air high rates of assimilation occur notwithstanding the small concentration of CO_2 present in the atmosphere.

2*a*. In the experiments with *Helianthus annuus*, which agree very closely among themselves, *leaves which remained turgid, and so kept their stomata*

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* *Loc. cit.*

† By these experiments and others in which he forced assimilation with excess of CO_2 for several days, Müller also concluded that Monocotyledons (or rather sugar-leaves) reach their maximum content of photosynthetic products more quickly, but that this maximum is much lower than in Dicotyledons (starch-leaves).

widely open, showed an average net rate of increase of nearly 17 *milligrammes per hour* in their dry weight per square decimetre. *Thus the result which Sachs obtained in a similar experiment* with detached leaves, 16·5 milligrammes, *is entirely confirmed.*

When a moderate allowance is made for assimilation of the CO_2 produced in respiration, the photosynthetic products reach a total of about 18 milligrammes per hour.

2*b*. The rate of production is not uniform throughout an experiment of seven or eight hours' duration ; it will, therefore, at times exceed the average rate and may reach more than 20 milligrammes per hour.

2*c*. When the stomata allow enough CO_2 for this assimilation to diffuse into the leaf, the internal leaf-temperature will probably be the limiting factor unless it exceeds 23° to 25° C. All the available evidence points to the conclusion that on a bright summer's day the leaf-temperature is much higher than this ; but the necessity for such high temperatures suggests the possibility that temperature limits the rate of assimilation more frequently than has usually been supposed. In this connection it would be of increased interest to know exactly how the stomata react to variations in leaf-temperature and illumination.

2*d*. The establishing of this high rate of open-air assimilation has the effect of decreasing the estimate of the proportion of available energy which is wasted by leaves of *H. annuus* on a bright sunny day. On such a day, so long as they are able to keep their stomata widely open, these leaves utilise about 30 per cent. of the photosynthetic radiation intercepted by them ; so that the waste is about 70 per cent., and possibly at times still less.

2*e*. When leaves of *H. annuus* lose their turgidity, their rate of assimilation is diminished. Some diminution occurs even when but a slight degree of limpness is perceptible to the eye or to the touch ; when the leaves are very flaccid, the horn hygroscope shows that their stomata are very little open, and they increase in dry weight very little, if at all.

2*f*. That Brown and Escombe observed no higher rates of assimilation in *H. annuus* than corresponded to an increase of about 5·5 milligrammes per hour is owing probably to several causes. In bright sunlight the temperature inside their glass leaf-cases rose unnaturally, if not injuriously, high, and the results of such experiments have little bearing on the question of the maximum rate of assimilation possible under natural conditions. In their experiments in moderate illumination, although the supply of radiant energy was in excess of the amount used in assimilation, it is probable that the stomata were not fully open.

The response of the stomata to different intensities of light seems to offer the most satisfactory explanation of this: thus *even very bright diffuse light appears to be insufficient to induce the stomata of* H. annuus *to open to their full extent.* It is suggested that this depends, not on the photosynthetic value of the radiation, which may be great, but on some other quality, such as its relative poverty in heat rays.

3. Compared with *Helianthus, Catalpa bignonioides* assimilates at much lower rates, a fact which is correlated with the absence of stomata from the upper surface of its leaves. Under conditions which enable leaves of *Helianthus* to increase in dry weight by 17 milligrammes per square decimetre per hour, *Catalpa* leaves show an increase of 5 or 6 milligrammes per hour.

Experiments with *Catalpa*, unlike those with *Helianthus*, have not given results far above those which Brown and Escombe obtained with the same plant. Brown and Escombe's more normal values with this plant indicate that its leaves stand exposure in a glass case better, so that they were able to carry out an experiment in intermittent sunlight. In my most successful experiment, however, the average result for 12 leaves exceeds their highest result; and if differences in the stomatal apertures of different leaves are taken into account, individual leaves must have increased in weight still more rapidly.

4. The high results obtained in the experiments with both *Helianthus* and *Catalpa* have been given by leaves detached from the plant. Whether leaves still attached to the plant assimilate at equally high rates will depend upon the stomata. The conditions differ in one important respect, since attached leaves have in general to absorb water from the stem against a negative pressure. After detachment the resistance has no longer to be overcome, and, until the vessels become blocked by air or mucilage, detached leaves might well open their stomata more widely than similar leaves still on the plant.

The magnitude of this effect will vary, however, with different plants, and under different conditions. The most marked effect would be expected in the case of trees; but with some, especially herbaceous plants, the water supply might be so abundant that even attached leaves could open their stomata to their widest extent.

Thus Brown and Escombe observed that much more CO_2 diffused into detached leaves of *Catalpa* than into leaves still upon the tree, but the same phenomenon would not necessarily have been observed with *Helianthus*. The few observations which have been made with the horn hygroscope on leaves of the latter have not revealed differences in respect of stomatal aperture between leaves on and off the plant.

5. It follows that the lower results obtained by Sachs and by Brown and

Morris with attached than with detached leaves of *H. annuus* cannot be entirely attributed to less widely opened stomata; but that part of the products of photosynthesis must have been translocated.

Broocks' experiments with the sugar-beet have proved that in that plant translocation does proceed during the day. On two continuously sunny days he found that leaves increased rapidly in dry weight till noon, and then, suddenly ceasing to assimilate, lost weight continuously at a uniform rate till about midnight. In this case also it seems more reasonable to suppose that in the morning translocation was taking place, though masked by assimilation, rather than that it only began when the leaves stopped assimilating.

I hope myself to obtain more *conclusive* evidence as to whether assimilation and translocation proceed concurrently. Now that a satisfactory modification of the dry weight method has been elaborated it is to be expected that this and other similar problems will be successfully attacked.

Receptors and Afferents of the Third, Fourth, and Sixth Cranial Nerves.

By Frances M. Tozer, B.Sc., and C. S. Sherrington, D.Sc.

(Received May 18,—Read June 6, 1910.)

The view generally accepted regarding the functions of the third, fourth, and sixth cranial nerve pairs is that they are purely motor. Certain experiments and observations made by one of us* and published some years ago threw doubt, however, on this belief.

It was then shown that severance of the third or fourth or sixth nerves at origin from the brain produces degeneration of practically all the nerve-fibres of the respective muscles innervated by those nerves and of the receptive endorgans with which those muscles are, as was shown,† plentifully supplied.

Further, on the physiological side it was found (1) that intracranial severance of both nn. trigemini in the monkey resulted in no obvious impairment or ataxy of eyeball movements, and (2) that severance of both nn. trigemini, combined with severance of both optic nerves, even after transection of the encephalic bulb, does not obviously depress the tonus of the eye-muscles (extrinsic). Since severance of the afferent nerves of muscles does almost universally depress their tonus severely, the inference was drawn that in the case of the extrinsic eye-muscles their tonus is not dependent on trigeminus or opticus, and may, therefore, be referable to afferents contained within the third, fourth, and sixth nerve-pairs themselves.

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* 'Physiol. Soc. Proc.,' June, 1894; 'Journ. of Physiol.,' vol. 17, p. 20; 'Roy. Soc. Proc.,' Feb., 1897, vol. 61, p. 247; *ibid.*, Sept., 1898, vol. 64, p. 120.

† Sherrington, 'Roy. Soc. Proc.,' Feb., 1897.

To examine further the whole question, the experiments and observations forming the basis of the present communication have since been undertaken.

A. *Characters of Nerve-endings and Nerve-fibres Normally Present in the Extrinsic Eye-muscles.*—Renewed examination has been made of the intramuscular nerve-twigs and nerve-endings of the normal eye-muscles of rabbit, cat, and monkey. For this purpose use has been made chiefly of the *intravitam* methylene blue method of Ehrlich and of osmic acid for staining and fixation prior to weak dissociation and teasing. In carrying out the methylene blue method the procedure recommended by Eugling* from experience in F. B. Hofmann's laboratory has been followed.

This examination of the muscles has fully confirmed the previously reported abundance of nerve-fibres distributed quite outside the region of distribution of motor plates, and that of these fibres many pass into the tendon of insertion and then recurve to end at junction of tendon with the fleshy part of the muscle.

The methylene blue preparations have given results agreeing with those obtained by Huber† and by A. Dogiel.‡ They show the whole region of the musculo-tendinous junction of the muscle to contain a zone rich in nerve-endings of obviously receptive (sensorial) type, extending as a band right across the entire transverse breadth of the muscle (see figure). In addition to these endorgans at tendon-muscle junction there are numbers of other nerve-endings of various and evidently non-motor type scattered throughout the muscles. Instances of these have been classified and figured by Huber and Dogiel.§ Our preparations confirm their description, and add little to it, except that we find, especially in the monkey, numerous endings which we incline to regard as receptive spindles (muscle-spindles) of peculiarly simple form.

Tendon Nerves.—In the normal eye-muscles it is usual by the osmic acid method to find in each muscle 250 to 350 small nerve-fibres entering the tendon from the fleshy part of the muscle. These fibres enter commonly in groups comprising three to eight fibres in a bundle. The individual fibres dichotomise, exhibit relatively thick, loose primitive sheaths, and nodes recurring frequently at short intervals. The entrance into the tendon of such numbers of myelinate nerve-fibres, obviously from their microscopic characters about to end, supplies a criterion for judging the afferent innervation of the muscle, and we have used it as such.

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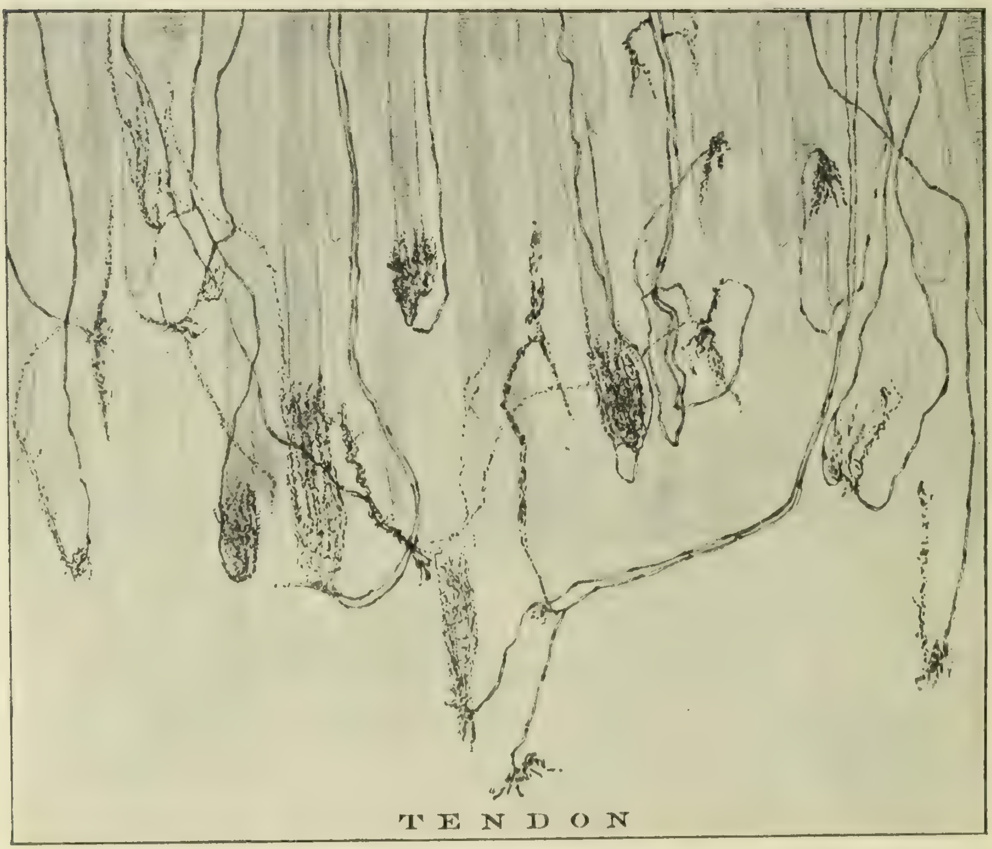
* 'Archiv. f. d. gesammt. Physiol.,' vol. 121, p. 279, 1908.

† 'Journ. of Comparative Neurology,' May, 1900, vol. 10, No. 2.

‡ 'Archiv f. mikroscop. Anatomie,' 1906, vol. 68.

§ *Ibid.*



Superior Rectus of Monkey (*Macacus rhesus*). Portion of the junction of the fleshy part of the muscle with its tendon, embracing about one-twelfth of the whole breadth of the muscle, showing tendon-nerves passing from the former into the latter, and then recurving to terminate in variously brush-shaped endings. *Intra-vitam* methylene blue method (Ehrlich). These nerves and endings disappear after intracranial severance of the 3rd nerve.

B. *Condition of the Orbital Nerves and Muscles after Severance of the Various Cranial Nerves Supplying the Orbit.*—To determine the source or sources of the afferent nerve-fibres of the several extrinsic ocular muscles we have had recourse to the degeneration method. Our experiments of this kind fall under six headings.

I. *Severance of the Third Cranial Nerve at its Origin from the Peduncle of the Cerebrum.*—Eleven experiments, including five with Dr. E. E. Laslett; one rabbit, three cats, seven monkeys. The time allowed for degeneration has varied between 5 days at shortest and 56 days at longest. During life the symptoms were the ptosis, external strabismus, and the dilatation of the pupil well known to result from paralysis of the third nerve.

Degeneration Results. (1) *Orbital Nerves.*—The nerve-trunks proceeding to and entering the superior, inferior, and internal recti muscles and the inferior oblique exhibited complete degeneration except for the fact that certain minute myelinate fibres sparse in number were quite sound. These fibres vary in diameter between 2 μ and 7 μ. Their number never exceeded 25 in any one of the above muscle nerves: commonly there were from 6 to 12 of them for each muscle; in some cases, in some of the muscle nerves, none at all were discoverable. The fibres resemble in size and appearance those of the short ciliary nerves proceeding from the ciliary ganglion. The fourth and sixth nerves, the branches of the ophthalmic division of the fifth nerve, and the short ciliary nerves were entirely free from degeneration.

(2) *Orbital muscles.*—In the superior, inferior, and internal recti muscles and in the inferior oblique no intramuscular nerve-endings either of motor or receptive (sensorial) type were found. At the tendon ends of these muscles, in most cases, though not in all, there persisted a few undegenerate nerve-fibres varying in diameter from 2 μ to 5 μ; the greatest number found in any one muscle was 13 (in inferior oblique). In two instances there were found Golgi tendon-organs, one to three in number. In the control muscles from the same orbit, namely external rectus and superior oblique, as also in the muscles of the orbit of the opposite side, the usual abundance of receptive nerve-endings as well as motor plates were visible, and the usual abundance of tendon nerves.

II. *Intracranial Severance of First Division of Fifth Nerve just Distal to Gasserian Ganglion.*—One experiment (monkey). The period allowed for degeneration was 15 days. The symptoms observable were anæsthesia of the cornea, upper eyelid, and forehead. The pupil of the operated side was the smaller.

Degeneration Results. (1) *Orbital Nerves.*—In the frontal and nasal branches (the lachrymal was not examined) of the fifth nerve no sound nerve-fibres remained. In the branch from third nerve to internal rectus muscle all nerve-fibres were sound: but the branch from third to inferior oblique contained three degenerated fibres and the branch to superior rectus contained seven degenerated fibres. The fourth nerve showed a considerable number of degenerated fibres, and so likewise did the sixth nerve.

(2) *Muscles.*—The muscles supplied by the third nerve exhibited no departure from the normal, either in respect to their intra-muscular nerves, their tendon nerves or their nerve-endings of receptive (sensorial) and motor type respectively. In the superior oblique and external rectus a number of receptive nerve-endings of normal appearance were seen and many normal tendon nerves. In this experiment the partial degeneration of fourth and sixth nerves was, it can scarcely be doubted, due to accidental partial injury of them at the time of severance of the ophthalmic division of the fifth, close to which they lie. And this supposition is abundantly supported by the results of the experiments mentioned in succeeding sub-sections. The fibres, extremely few in number, found degenerate in certain of the branches of third may have come from the ophthalmic division of fifth.

III. *Intracranial Severance of Fourth Nerve, and also of Ophthalmic Division of Fifth Nerve.*—Two experiments (monkey). Period allowed for degeneration, 28 and 50 days respectively.

Degeneration Results. (1) *Orbital Nerves.*—In the frontal and nasal branches of fifth nerve a few fibres (maximum 10) of small diameter were undegenerate, otherwise their degeneration was absolute. The fourth nerve was completely degenerate, no sound fibres remaining. The sixth nerve and the branches of the third nerve contained no degenerate fibres, except that the branch from the inferior division of third to the inferior oblique muscle exhibited 10 degenerate fibres.

(2) *Muscles.*—In superior oblique muscle no nerve-endings were found, and in one case no sound tendon nerves at all; in the other case, five sound fibres of minute diameter were found in the tendon. All the muscles, except superior oblique, exhibited the usual nerve-endings, and the usual number of tendon nerves perfectly sound.

IV. *Intracranial Severance of Ophthalmic Division of Fifth Nerve and of the Sixth Cranial Nerve.*—Three experiments, including one with Dr. E. E. Laslett (monkey). Symptoms as in II, combined with external strabismus. Periods allowed for degeneration, 15, 16, and 50 days respectively.

Degeneration Results. (A) In the 16-day experiment. (1) *Orbital Nerves.*—No sound fibres were detected in the nasal, frontal, and lachrymal branches of fifth nerve, nor in the sixth nerve at its entrance into the orbit. Conversely, in the branches of the third nerve and fourth nerve, no nerve-fibres at all were degenerate.

(2) *Muscles.*—In external rectus, although all nerve-endings had dis-

appeared, about 20 nerve-fibres of minute diameter remained sound, three reaching the tendon. In the other muscles, the nerve-endings and tendon nerves were normal.

(B) In the 15-day experiment the results were the same as the above in regard to the branches of the fifth and the sixth nerves.

In the branch of the third to superior rectus, however, and in the fourth nerve, as it entered the superior oblique, there were degenerate fibres to the number of 70—80. From external rectus all nerve-endings had disappeared, but one sound nerve-fibre of small calibre was present, not, however, reaching the tendon. The remaining muscles of the orbit, including superior rectus and superior oblique, exhibited the usual normal nerve-endings and tendon nerves.

(C) In the 50-day experiment the results agreed with the above, except that in frontal nerve, at its exit from orbit, six minute nerve-fibres were still sound, and in sixth nerve, at entrance into external rectus, five minute nerve-fibres were still sound. In the tendon of external rectus, three minute sound fibres were discovered, otherwise, all nerve-endings and nerve-fibres had disappeared. In this experiment, as in the 16-day experiment, the fourth nerve and the branches of the third nerve were entirely free from any trace of degeneration.

V. *Intracranial Severance of Fourth Nerve, Ophthalmic Division of Fifth Nerve, and Sixth Nerve.*—One experiment (monkey). Symptoms as in IV. Period allowed for degeneration, 50 days.

Degeneration Results. (1) *Orbital Nerves.*—Complete degeneration of fourth nerve and of all branches of ophthalmic of fifth and the sixth nerve.

(2) *Muscles.*—In superior oblique no nerve-endings were found, but a few nerve-fibres persisted sound; some of these approached the tendon end. In external rectus no nerve-endings and no tendon nerves remained. In the remaining muscles of the orbit the customary nerve-endings and tendon nerves were discoverable as usual, and showed no departure from normal.

VI. *Intracranial Severance of Third Nerve, Ophthalmic Division of Fifth Nerve and the Sixth Nerve.*—One experiment (monkey). Period allowed for degeneration, 30 days. Symptoms during life were ptosis, practical immobility of eyeball, great dilatation of pupil, anæsthesia of cornea, upper lid and forehead.

Degeneration Results. (1) *Orbital Nerves.*—The frontal branch of fifth contained no sound fibres, the nasal branch contained two, and the lachrymal branch contained a few sound fibres of minute size.

The branch from third nerve to internal rectus contained no sound fibres, but the branch to inferior rectus contained six, that to superior rectus four, and that to inferior oblique sixteen sound fibres, all of minute size. The fourth nerve entering superior oblique showed a certain number of degenerate fibres, some large, but in the main the nerve was sound. The short ciliary nerves were found to be entirely free from degeneration.

(2) *Muscles.*—No nerve-endings were discoverable in any of the muscles except superior oblique, which latter showed them without any obvious departure from the normal. From the remaining muscles the nerve-endings had all disappeared, and the tendon nerves also were gone, though in the tendon of internal rectus one sound nerve-fibre persisted, and in that of superior rectus four, and in that of inferior rectus six.

(Regarding *levator palpebræ* in this and the other experiments we are reserving our remarks for a fuller account than the present communication allows.)

CONCLUSIONS.

After severance, therefore, of the third nerve at its origin, not only do the motor-plate endings disappear from superior, inferior and internal recti and inferior oblique muscles, but the musculo-tendinous and intramuscular receptive (sensorial) nerve-endings also disappear from these muscles, together with the afferent nerve-fibres supplying them.

Similarly with the superior oblique muscle after intracranial severance of the fourth nerve. Similarly with the external rectus muscle after intracranial severance of the sixth nerve.

The fifth nerve may send a few fibres to the muscles in the orbit, but this supply in rabbit, cat, and monkey is insignificant in amount.

A few sparse myelinate fibres of minute size ($2\,\mu - 5\,\mu$) still persist undegenerate in the extrinsic eye muscles after combined severance of the third, fourth, and sixth nerves, and of the ophthalmic division of the fifth; some of these pass into the tendons of the muscles. Their source appears to be the ciliary ganglion, and in the case of the inferior oblique muscle a small accessory ganglion lying in the nerve of that muscle, sometimes quite close to the entrance of the nerve into the muscle itself. The ciliary ganglion is, as Langley and Anderson* have shown, entirely an efferent relay station, and is the equivalent, as Gaskell† pointed out, of a sympathetic ganglion. That it contains no cells equivalent to those of the spinal root-ganglia seems certain from the absence of sound fibres in the intracranial stumps of third, fourth, or sixth nerves, distal to their intracranial severance. The few sparse minute myelinate fibres probably of ciliary source which, in the orbit, find their way into the orbital muscles, in many instances accompany blood-vessels, and in a small number of instances we have been able to trace some of them actually to termination in the walls of arterioles.

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* 'Journ. of Physiol.,' vol. 17, p. 184, 1894; *ibid.*, vol. 32, p. 173.

† 'Journ. of Physiol.,' vol. 10, p. 153, 1889.

The third, fourth, and sixth cranial nerve pairs are therefore afferent-efferent, their afferents belonging to the receptive (sensorial) endings with which all the extrinsic eye-muscles are richly provided. The afferent divisions of these cranial nerves are by their distribution exclusively proprio-ceptive,* supplying no other organs either extero-ceptive or intero-ceptive.

As regards the reflexes obtainable from these nerves our experiments have dealt chiefly with the branch from third nerve to inferior oblique. When this muscle after detachment is briskly stretched between ivory-tipped forceps reflex movements (of ear, etc.) are obtainable.

As regards the normal functions of these afferents of the eye-muscles, it was shown previously† that after the conjunctiva, both palpebral and ocular, and the cornea of both eyes have been rendered deeply anæsthetic by cocaine, the eyes can still be directed to any given point with considerable accuracy in a completely dark room. One must think that for such performance the exercise of some peripheral apparatus of sense is required. Under the above conditions, the superficial fifth and optic nerves having been excluded, the only possible channels seem the proprio-ceptive sense-organs and afferents of the third, fourth, and sixth nerve-pairs themselves.

The occurrence of degeneration of the third, fourth, and sixth‡ cranial nerves in cases of tabes, a disease defined as "primary progressive degeneration of the first afferent (sensory) projection system of neurones" (Mott),§ becomes less anomalous in view of these nerves being afferent-efferent, and not purely efferent. The ocular deviations common in tabes may be referable to loss of reflex tonus, owing to degeneration of the proprio-ceptive afferents rather than to actual paralysis from destruction of the motor fibres of these nerves.

The fuller consideration of these and other points we leave to be dealt with in a more complete account which we hope soon to furnish.

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* Sherrington, 'Integrative Action of the Nervous System,' p. 336, 1906.

† Sherrington, 'Roy. Soc. Proc.,' vol. 64, p. 120, 1898.

‡ Oppenheim and Siemerling, 'Archiv f. Psychiatrie,' vol. 18, p. 161, 1887.

§ 'System of Medicine,' vol. 7, p. 98, 1901 (Allbutt).

The Relation of Light Perception to Colour Perception.

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(Communicated by Prof. E. H. Starling, F.R.S. Received June 3,—Read June 30, 1910.)

(From the Institute of Physiology, University College.)

Since the majority of the theories of *colour* perception which have been propounded have been really theories of *light* perception, the two subjects, namely, colour and light perception, though really quite distinct, have become so interwoven in the discussion of the question that many seem quite incapable of distinguishing the two. Yet it may be easily shown that light perception and colour perception are quite distinct. In fact, we can divide cases of colour blindness into two classes, according as the defect is (*a*) one of light perception, or (*b*) one of colour perception or differentiation without any defect in light perception. Of course, both defects may be present in the same individual.

The investigation of these two classes of defective vision is much facilitated by the use of a spectrometer which I have devised for the purpose, and which is so arranged as to make it possible to expose to view in the eye-piece the portion of a spectrum between any two desired wave-lengths. It consists of the usual parts of a prism spectroscope, *i.e.* a collimator with adjustable slit, prism, and telescope with eye-piece of the following dimensions :—

Focal length of collimator and telescope object glasses = $7\frac{1}{8}$ inches (180 mm.).
Clear aperture of collimator and telescope object glasses = $\frac{7}{8}$ inch (22 mm.).
Slit, 7 mm. effective length of jaw, with wedge for reducing the effective length of the slit, protective cap, comparison prism, and screw adjustment for the slit width, with divided head.

The prism is of flint glass, 1·65 refractive index for D. Eye-piece, Ramsden form, focussing on to the shutters described below.

In the focal plane of the telescope are two adjustable shutters with vertical edges, the shutters being carried by levers which rotate about centres near the object glass of the telescope. The shutters can be moved into the field from right and left respectively, each by its own micrometer screw, and to each screw is attached a drum, the one being on the right and the other on the left of the telescope. On each of these drums is cut a helical slot in which runs an index, and the drum is engraved in such a manner that the reading of the index gives directly in wave-lengths the position in the spectrum of the corresponding shutter.

Thus it will be seen that if, for instance, the reading on the left drumhead is 5320 and that on the right drumhead is 5950, the region of the spectrum from λ 5320 to λ 5950 is exposed to view in the eye-piece.*

The instrument is used as follows:—As far as possible a known quality and intensity of light should be employed. A small oil lamp is quite suitable for the purpose. The observer should first ascertain the exact position of the termination of the red end of the spectrum, the left-hand shutter being moved across until every trace of red just disappears. The position of the pointer on the left-hand drum is noted, and the wave-length recorded. The left drum is then moved so that the shutter is more towards the middle of the spectrum. The right-hand drum is then moved until the pointer indicates the wave-length recorded as the termination of the red end of the spectrum. The observer then moves the left-hand shutter in and out until he obtains the largest portion of red which appears absolutely monochromatic to him, no notice being taken of variations in *brightness*, but only in *hue*. The position of the index on the left-hand drum is recorded. The left-hand shutter is then moved more towards the violet end of the spectrum, the right-hand shutter being placed at the position previously occupied by the left-hand shutter. In this way the whole of the spectrum is traversed until the termination of the violet end of the spectrum is finally ascertained with the right-hand shutter. The variation of the size of the patches and the termination of the spectrum with different intensities of light can be noted.

The instrument can also be used for ascertaining the exact position and size of the neutral patches in dichromics, the position of greatest luminosity, and the size and extent of pure colours. When it is used to test colour-blindness, the examinee should first be shown some portion of the interior of the spectrum, and then asked to name the various colours which he sees. In this way he will have no clue to the colours which are being shown him.

Tested with this instrument a normal individual will, as a rule, name six distinct colours (viz., red, orange, yellow, green, blue, violet), and will mark out by means of the shutters about 18 monochromatic patches. Occasionally we come across individuals with a greater power of differentiating hues, to whom, as to Newton, there is a distinct colour between the blue and violet, which Newton called indigo. Such individuals will mark out a greater number of monochromatic patches, from 22 up to 29. The limited number of monochromatic patches which can be marked out in this way is at first surprising when we consider how insensibly one part of the spectrum seems to shade into the next when the whole of the spectrum is looked at. The number and position of the patches present, however, great uniformity from one case to another.

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* This spectrometer has been constructed for me by Adam Hilger, Ltd., and was purchased with a grant made by the Government Grant Committee of the Royal Society.

I propose to deal with a certain number of cases of defective colour vision as investigated by this instrument, and to show how we may differentiate those defects due to failure of light perception from those due to failure of colour or hue perception.

1. *Defective Light Perception with Normal Colour Perception.*

As an illustration of this class I may mention a case I have recently examined in which there was shortening of the red end of the spectrum with absolutely normal hue perception. A boy wishing to enter the Navy had been examined with the Holmgren test and certified to have normal colour perception. He was rejected, however, with the lantern test. He was then sent to me. I found his hue perception quite normal. He matched and named colours with ease and accuracy. When tested with the spectrometer, however, I found that his spectrum for bright light was shortened to λ 700, the limit for normal individuals being about λ 760 to λ 780. He had also defective perception for the red rays adjacent to the shortened portion. When examined with my lantern* he failed altogether to see the standard red light No. 2 at about 20 feet distance, though he could clearly see the aperture through which the light came. The bright red light consisting almost exclusively of rays from λ 625 to λ 731 was not even visible to him as light.

In the latest reports of the Board of Trade there are several cases of men who have passed the green test of Holmgren, but have failed with the rose test and have therefore been designated completely red-blind. These cases are probably similar to the boy whose condition I have just described. Such cases are undoubtedly red-blind for the rays which are not seen, but not in the sense of the Young-Helmholtz or Hering theories. This boy was in a similar position to a person who is unable to hear very low notes on an organ. Such persons may have absolutely normal perception of tone differences above a certain number of vibrations per second, and in the same way these cases of so-called red blindness may have a normal appreciation of colour differences for all the spectrum except the least refrangible rays, which do not influence their retinas at all. In the same way, just as certain individuals may be deaf to the highest notes of music, so there is a class of persons whose spectrum is shortened at the violet end. Although such cases will have an appreciation of colour differences as good as that of the average individual, their colour sense will not be identical with the latter.

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* This is described in my book on 'Colour Blindness and Colour Perception' (International Scientific Series), 1909, p. 264.

This will be evident if we consider the influence of a shortened spectrum upon colour vision. The first evident fact is that bodies reflecting only light, the rays of which occupy the missing portion of the spectrum, appear black. Nearly all colours are compound, that is to say, the coloured body reflects other rays than those of the colour seen. Thus a blue-green glass may transmit the green, blue, and violet rays of the spectrum. Let us suppose that we have a substance reflecting the green, blue, and three-quarters of the violet, the colour of the body to a normal person being green. Then if we had another substance which reflected the whole of the violet, it would appear blue. But with a person who could not perceive the terminal fourth of the violet the colour would look exactly the same as the green one, and as he could not distinguish between the two, he would be in continual difficulty with blues and greens. All coloured objects reflecting rays occupying the missing portion appear darker than they do to the normal sighted, and are always matched with darker colours belonging to a point more internal. Thus a dichromic with a shortened red end of the spectrum matches a red with a darker green.

It will be noticed that a shortened spectrum, especially if one end only be affected, may interfere very little with the general appreciation of shade. If, for instance, we take a case in which the red end of the spectrum is shortened, so that only three-quarters of the red of the normal sighted is seen, then all bodies which equally reflect or transmit these rays can be correctly compared, because a similar portion of light has been removed from each. It is only when one colour reflects or transmits the rays occupying the shortened portion and the other does not that there is any definite interference with the appreciation of shade. Again, if neither colour reflects or transmits rays occupying the shortened portion of the spectrum, there will obviously be no interference with the appreciation of shade.

A very common mistake due to shortening of the red end of the spectrum is the confusion of pink and blue. If a person with considerable shortening of the red end of the spectrum is shown a pink which is made up of a mixture of red and violet, the red consisting of rays occupying the missing portion of the spectrum, only the violet is visible to him, and so the pink appears a violet without a trace of red. This pink is therefore matched with a violet or blue very much darker than itself.

Mistakes which are due to shortening of the spectrum may be remedied if we subtract the rays occupying the missing portion from the colour of confusion. For instance, if we take a blue and a pink which have been put together as identical by a person with a shortened red end of the spectrum, and look at them through a glass which is opaque to the red but transparent to the remaining rays of the spectrum, both will appear alike in hue and shade. A person with considerable shortening of the red end of the spectrum will look at a red light (which is so dazzlingly bright to a normal-sighted person as to make his eyes ache after looking at it closely for a few seconds), at a distance of a few inches, and remark that there is nothing visible, and that the whole is absolutely black. It is obvious that the light must consist only of rays occupying the missing portion of the spectrum.

The same remarks which I have made for a shortened spectrum apply to cases in which there is defect of light perception through absorption or any other cause. The person having the defect is placed in a similar position to a normal-sighted person with those particular rays removed or reduced to the same intensity.

Another effect of shortening of the spectrum when it is sufficient to interfere with the difference perception which appears to be inherent in the central nervous system is that the colours appear to be moved in the direction of the unshortened portion. For instance, we find the neutral point of the dichromic with shortening of the red end of the spectrum further towards the violet end of the spectrum in comparison with a case in which the spectrum is of normal length. In the same way a trichromic with a shortened red end of the spectrum has the junction of the red and green nearer the violet end than in a case where there is no shortening.

The point that I specially wish to emphasise is that, though every case in which there is defective light perception can be explained by a defective sensibility to light of certain wave-length, not a single case of the very large number of persons that I have examined can be explained on the older theories, that is, the defect of light perception cannot be explained on the assumption that there is a defect in a light-perceiving substance which is sensitive to rays of light from a considerable range of the spectrum.

2. *Cases in which there is Defective Colour Perception.*

Even in cases where there is actual colour blindness or deficiency of hue perception it is equally important to differentiate between the perception of light and the perception of hue. Let us take as example a case which at first sight appears to be in support of the older theories, a case of so-called red blindness, in which there is dichromic vision with considerable shortening of the red end of the spectrum. It can be easily shown that the defect which has caused the non-perception of certain red rays has not caused the dichromatism. A case of this kind will put a bright rose with a dark blue, a bright red with a dark green, designate a dark red leather as black, and find difficulty in seeing in a red light. On examining such a case it will be found that the shortening of the red end of the spectrum may be abrupt or more or less diffuse, that is, there may be defective perception of red over a much larger area. It will, however, be found that the light perception is absolutely normal immediately adjacent to the portion in which there is defective light perception. For instance, the red may be shortened to λ 680; at λ 670 the perception of red may be defective to about half the normal, and at λ 660 it may be quite normal. If we now test such a case with spectral colours from λ 660 onwards to the violet end of the spectrum, we find that their luminosity, *i.e.*, differences of light and shade, is identical with the normal. Thus the subtraction of the element which causes the non-perception of certain rays cannot be responsible for the dichromic vision which extends from λ 660 to λ 385.

In estimating defects of light perception, colours should be directly compared in order to ascertain their comparative luminosity. For instance, light of λ 589 can be compared with light of λ 570 and λ 535. A comparison with white light gives rise to results which are very fallacious. Not only have the missing red rays to be subtracted, but if the individual have a spectrum which is lengthened at the violet end or is more sensitive to any other rays than the normal, a co-efficient corresponding to these rays must be added. Therefore if α represent the missing red rays and β the added violet rays, the formula of white light as seen by the individual in question, as compared with the normal, will be white $-\alpha+\beta$.

Still more difficult to explain on any light perception theory are the cases of so-called green-blindness. These, as I have shown, are simply cases of dichromic vision without shortening of the spectrum, and, indeed, with no defect in light perception in any part of the spectrum. If to such cases we give colours to compare which differ only slightly in shade but are absolutely different in hue, we find that their selection of the lightest or darkest corresponds to that which would be made by a normal individual. A red and a green, or an orange and a green, will be selected which to the normal eye match exactly in shade.*

During two years I examined every case of colour-blindness which came to my notice with Rayleigh's colour mixing apparatus, in addition to other methods. In this apparatus a match is made between λ 589 (sodium yellow) and a mixture of λ 670 (lithium red) and λ 535 (thallium green). The results obtained, however, seem to be quite independent of the power possessed by the individuals examined to appreciate differences in colour. In fact, by this apparatus we may obtain abnormal values in persons who have apparently no defect in hue perception, while others who are undoubtedly colour-blind may give results which correspond to that given by the average individual. The results seem to be determined not by varying power of appreciation of hue, but by slight variations in the luminosity curve of different parts of the spectrum in different individuals. Another important factor, probably the chief one, is the state of adaptation of the eye at the time of the observation.

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* These observations are in agreement with those of A. König, who also found that there were considerable variations in the luminosity curve of persons of normal colour perception, whilst a so-called green-blind had almost exactly the same curve as himself and a normal sighted woman. 'Beiträge zur Psychologie und Physiologie der Sinnesorgane,' 1891, p. 311.

All who have had practical acquaintance with colour-blindness are aware that all dichromics are not equally colour-blind; one will be readily detected by almost any test, whilst another requires the greatest care to detect at all. I find that the cases of dichromic vision vary from almost total colour-blindness to cases bordering on those I have called the trichromic. I give below the monochromatic patches marked out by four dichromics with my spectrometer in similar conditions. The light was 180 meter-candles, the slit the same size in each, and the eyes light-adapted. The size of the patches is measured in micro-millimetres ($\mu\mu$).

No. 1 cannot see any difference between the cactus flower and its leaf, and does not see any difference between the red and green signal lights except at the moment when there is a change from one to the other. It is obvious that this case is much more colour-blind than the ordinary dichromic. On the other hand, a case bordering on the trichromic will see about eight definite patches in the spectrum; make a normal match with Rayleigh's apparatus; pass the Holmgren test with ease; and play pool regularly without on any occasion mistaking the coloured balls. Such a case, when examined with the spectrometer, has eight monochromatic patches instead of the normal eighteen; says that there are only two colours in the spectrum, red and violet, and has a very small neutral patch. It is quite easy to show that such a case is dichromic, as he will match spectral blue-green with a mixture of spectral red and violet: $\lambda\,670 + \lambda\,470 = \lambda\,490$. How could a defect in a light-perceiving substance have such varying results? If we assume that in these cases there is a varying perception of colour difference, the facts are explained.

It is stated in favour of the Young-Helmholtz theory that with three variables we can produce innumerable variations, the fact that on trial we

Case.	Monochromatic patches.			Remarks.
	No.	λ.	μμ.	
1	1	665	167	
	2	498	69	Patch of greatest luminosity, λ 571—608.
		429		Neutral patch, λ 4854—5078.
2	1	635	132	
	2	503	9	Neutral patch, λ 4975—5100.
	3	494	12	
	4	482	67	
		415		
3	1	735	130	
	2	605	71	Neutral patch, λ 5005—5185.
	3	534	35	
	4	499	7	
	5	492	10	
	6	482	68	
		414		
4	1	775	122	
	2	653	136	Patch of greatest luminosity, λ 587—642.
	3	517	11	
	4	506	12	Largest neutral patch, λ 504—515.
	5	494	10	
	6	484	11	
	7	473	57	No defect of light or shade perception.
		416		I agreed with all his selections.

can only perceive a limited number of variations in the spectrum being entirely ignored. The same argument would, however, apply to a two-colour theory, since we can make innumerable variations with only two variables. A more important argument, however, against the three-colour theory is to be found in the fact that there is a whole group of cases which possess three definite colour sensations and yet are undoubtedly colour-blind. These I have called the class of trichromics. Such persons may match the Holmgren wools perfectly, though they may make mistakes in *naming* single wools. A very frequent mistake when shown a single skein of yellow wool is to call it red or green, while a blue wool they will call green or violet. Similar mistakes are made with a lantern. When examined with the spectrometer, they will name only three colours as against the six named by an ordinary individual. When questioned, they regard the application of a special name of yellow or blue to definite parts of the spectrum as word-splitting, and would regard the words reddish-green or greenish-violet as more appropriate to the parts of the spectrum in question.

I give below the details of three trichromic cases as examined by the spectrometer.

Monochromatic Patches in Wave-lengths as Marked out by Trichromics 1, 2, and 3.

No. of patches.	Case 1.		Case 2.		Case 3.	
	λ.	δλ.	λ.	δλ.	λ.	δλ
1	7341 6160	1181	7604 6149	1455	7604 6105	1499
2	6037 5877	160	6050 5877	173	6032 5877	155
3	5873 5677	196	5883 5694	189	5877 5765	112
4	5679 5408	271	5694 5453	241	5765 5660	105
5	5411 5309	102	5446 5166	280	5660 5529	131
6	5309 5178	131	5166 4991	175	5529 5323	206
7	5176 5070	106	4991 4916	75	5326 5024	302
8	5070 4969	101	4921 4855	66	5024 4855	169
9	4972 4806	166	4850 4713	137	4850 4713	137
10	4811 4019	792	4713 3853	860	4713 3853	860
	Length of spectrum, 3322 Angström units.		Length of spectrum, 3751 Ångström units.		Length of spectrum, 3751 Ångström units.	

δλ represents the size of the monochromatic patch in Ångström units.

On comparing these three cases, it will be noted that there is a series of very remarkable coincidences. Each presents 10 monochromatic patches, and in each there are two wide patches at the extremities and one in the centre. It will be noticed also that the smaller patches are grouped round this central one. In 1 and 3 there are in each case five intermediate patches on one side and two on the other, but in 1 the five patches are on the violet side and in 3 on the red side.

As far as these intermediate regions are concerned, 3 looks like a reversal of 1. No. 2 has four patches on one side and three on the other. There are also coincidences in the wave-lengths. The first two patches on the violet side in 2 and 3 are exactly alike in size and position. All three coincide at λ 5877. Nos. 2 and 3 have a spectrum of normal length and of exactly the same size, whilst 1 has a spectrum which is shortened at both ends but more shortened on the red side. The centre of the smallest patch of 1 is λ 5543, and the centres of 2 and 3 λ 5306 and λ 5174 respectively. It would appear as if these were three similar cases with different absorption.

We know that the portions of the spectrum which differ are those which are most influenced by the varying pigmentation of the yellow spot. It will be noticed that the total effect is the same, the spectrum being divided into 10 patches in each case, so that what is gained on one side is lost on the other. No. 2 is the spectrum of Sir J. J. Thomson, further details of whose colour perception I have given in the 'Proceedings of the Royal Society,' B, vol. 76, 1905, p. 194. Sir J. J. Thomson, when making the match previously mentioned with Rayleigh's apparatus, put more green than the normal in the mixed colour, but the other two trichromics made the normal match. It is obvious that this alteration in light perception could not produce the defect in colour perception which was found.

By the use of the spectrometer described above, it is easy to detect cases of defective colour perception of a smaller degree than the two classes I have just described. In fact, if we regard the normal individual as hexachromic, we can differentiate classes possessing five and four colour sensations respectively, which may be called pentachromic and tetrachromic. In these cases, the only loss is the power of differentiation of hue. The sensibility to light, *i.e.* to luminosity, may be normal throughout the whole spectrum. These minor cases will, as a rule, match wools perfectly, but may make slight mistakes when asked to name the colours of single skeins. The pentachromic may be regarded as differentiating the colours red, yellow, green, blue, violet, while the tetrachromic would name only the colours red, yellow, green, violet. It is evident that none of these classes can be explained by assuming the absence of any substance or set of percipient elements sensitive to light over a considerable area of the spectrum. Any theory of colour vision must explain the position of the colours and the varying size of the monochromatic patches in each case.

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ON SCANDIUM.—PART II.—By Sir William Crookes, D.Sc., LL.D., For. Sec. R.S. (Abstract) 79

ON THE ROTATORY CHARACTER OF SOME TERRESTRIAL MAGNETIC DISTURBANCES AT GREENWICH AND ON THEIR DIURNAL DISTRIBUTION. By Robert B. Sangster 85

ON THE DIRECTION OF MOTION OF AN ELECTRON EJECTED FROM AN ATOM BY ULTRA-VIOLET LIGHT. By R. D. Kleeman, D.Sc., B.A., Mackinnon Student of the Royal Society; Emmanuel College, Cambridge 92

ON THE ABSORPTION OF GASES BY CHARCOAL. By Miss I. F. Homfray, D.Sc. 99

FLOW OF WATER IN CURVED PIPES. By John Eustice, B.Sc., Professor of Engineering, Hartley University College, Southampton 107

OBITUARY NOTICES OF FELLOWS DECEASED: Sir Benjamin Baker; Edward John Routh; Dmitri Ivanovitch Mendeléeff; George Gore; Julius Thomsen; William James Russell; Simon Newcomb .. .. i—xxxviii

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PROCEEDINGS
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Series B. Vol. 82. No. **B 558.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page
Trypanosome Diseases of Domestic Animals in Uganda.—I. Trypanosoma Pecorum. (Plates 11 and 12) 468
Experiments to ascertain if Cattle may act as a Reservoir of the Virus of Sleeping Sickness (Trypanosoma gambiense) 480
"Muhinyo," a Disease of Natives in Uganda 485
The Natural Food of Glossina palpalis 490
Mechanical Transmission of Sleeping Sickness by the Tsetse Fly . . 498
By Colonel Sir DAVID BRUCE, C.B., F.R.S., Army Medical Service; Captains A. E. HAMERTON, D.S.O., and H. R. BATEMAN, Royal Army Medical Corps; and Captain F. P. MACKIE, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908–10.)

The Chromaphil Tissues and the Adrenal Medulla. By SWALE VINCENT, M.D., D.Sc., F.R.SS. E. and C., Professor of Physiology in the University of Manitoba. (Plate 13) 502

On the Occurrence of a Mesocœlic Recess in the Human Brain, and its Relation to the Sub-Commissural Organ of Lower Vertebrates; with special reference to the Distribution of Reissner's Fibre in the Vertebrate Series and its possible Function. By ARTHUR DENDY, D.Sc., F.R.S., Professor of Zoology in King's College (University of London), and G. E. NICHOLLS, B.Sc., Assistant-Lecturer and Demonstrator in Zoology in King's College. (Plate 14) 515

Autotoxæmia and Infection. By E. C. HORT, F.R.C.P. Ed., Assistant Physician to the Italian Hospital 529

The Blood Volume of Mammals as Determined by Experiments upon Rabbits, Guinea-pigs, and Mice, and its Relationship to the Body Weight and to the Surface Area expressed in a Formula. By GEORGES DREYER, M.A., M.D., and WILLIAM RAY, B.Sc., M.B. (Abstract) . . 545

PRINTED FOR THE ROYAL SOCIETY AND SOLD BY
HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price Five Shillings and Sixpence.

No. B 558.

August 10, 1910.

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λ 5877. Nos. 2 and 3 have a spectrum of normal length and of exactly the same size, whilst 1 has a spectrum which is shortened at both ends but more shortened on the red side. The centre of the smallest patch of 1 is λ 5543, and the centres of 2 and 3 λ 5306 and λ 5174 respectively. It would appear as if these were three similar cases with different absorption.

We know that the portions of the spectrum which differ are those which are most influenced by the varying pigmentation of the yellow spot. It will be noticed that the total effect is the same, the spectrum being divided into 10 patches in each case, so that what is gained on one side is lost on the other. No. 2 is the spectrum of Sir J. J. Thomson, further details of whose colour perception I have given in the 'Proceedings of the Royal Society,' B, vol. 76, 1905, p. 194. Sir J. J. Thomson, when making the match previously mentioned with Rayleigh's apparatus, put more green than the normal in the mixed colour, but the other two trichromics made the normal match. It is obvious that this alteration in light perception could not produce the defect in colour perception which was found.

By the use of the spectrometer described above, it is easy to detect cases of defective colour perception of a smaller degree than the two classes I have just described. In fact, if we regard the normal individual as hexachromic, we can differentiate classes possessing five and four colour sensations respectively, which may be called pentachromic and tetrachromic. In these cases, the only loss is the power of differentiation of hue. The sensibility to light, *i.e.* to luminosity, may be normal throughout the whole spectrum. These minor cases will, as a rule, match wools perfectly, but may make slight mistakes when asked to name the colours of single skeins. The pentachromic may be regarded as differentiating the colours red, yellow, green, blue, violet, while the tetrachromic would name only the colours red, yellow, green, violet. It is evident that none of these classes can be explained by assuming the absence of any substance or set of percipient elements sensitive to light over a considerable area of the spectrum. Any theory of colour vision must explain the position of the colours and the varying size of the monochromatic patches in each case.

Trypanosome Diseases of Domestic Animals in Uganda.
I. Trypanosoma Pecorum.

By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, 1908–9.)

(Received May 13,—Read June 16, 1910.)

[Plates 11 and 12.]

Synonyms.

Trypanosoma dimorphon (Dutton and Todd).
Trypanosoma congolense (Broden).
Trypanosoma confusum (Montgomery and Kinghorn).
Dr. Edington's trypanosome from Zanzibar (Bruce, Hamerton, and Bateman).
Trypanosome from Chai-Chai, Zambesi, Zululand (Theiler).
Trypanosome from Southern Rhodesia (Bevan).

Introduction.

As might be expected from the tropical nature of the country, Uganda suffers much from protozoal diseases, and as the wealth of the natives consists principally in cattle, goats, and sheep—the King and chiefs having huge herds—there is much loss.

To give an idea of the enormous mortality which must take place among the herds of cattle in Uganda, Sir Apolo Kagwa, K.C.M.G., the Prime Minister, may be quoted. He informed the Commission that during 1908 he had 1396 cows, which had 2021 calves, and of these calves 709 died—35 per cent. Further, of cows, bulls, and full-grown bullocks, there had died 351. This makes a total of 1060 deaths in one year. The number of the whole herd is not given, but may be put down at 4000. This means a yearly death-roll of about 25 per cent. During the same year the Government Transport Department in Entebbe lost 156 oxen between June and November.

During 1909 the Commission had the opportunity of investigating several outbreaks of trypanosome disease among domestic animals, one among the transport cattle at Entebbe (lat. 0° 3′ N., long. 32° 30′ E.); another at Kampala (lat. 0° 18′ N., long. 32° 35′ E.); a third at the Uganda Company's estate at Namukekera (lat. 0° 40′ N., long. 32° 17′ E.); a fourth at Mr. Walsh's farm at Kabula Muliro, a few miles west of Namukekera; and a fifth at the Mabira Rubber Estate (lat. 1° 50′ N., long. 32° 40′ E.).

The commonest trypanosome disease among cattle in Uganda is caused by a trypanosome of the *dimorphon* type, which it is proposed to call *Trypanosoma pecorum.* This species is probably the same as that which has been known by the name *Trypanosoma dimorphon* (Dutton and Todd), and is either identical with, or very similar to, Broden's *Trypanosoma congolense.* The name *dimorphon* is a misleading one, and can only be accounted for by Dutton and Todd describing under one name two species of trypanosomes occurring in the same animal. No one, so far as we are aware, has re-discovered Dutton and Todd's *Trypanosoma dimorphon,* although many attempts to reconcile later observations with theirs have been made. Certainly the strain kept at the Liverpool School of Tropical Medicine under that name does not agree with the original description. The term *Trypanosoma dimorphon* must therefore disappear, since it was born of a misapprehension. But it will at once be said, if the name *Trypanosoma dimorphon* must go, why not call the species *Trypanosoma congolense?* The only reasons that can be given are, that *Trypanosoma congolense* is a local name and, therefore, not very suitable, and secondly, that if it comes to strict priority, then *Trypanosoma dimorphon* holds the field in spite of its misleading character and the error its authors fell into, because it cannot be denied that there is a strong feeling that *Trypanosoma dimorphon* and *Trypanosoma congolense* are one. At the same time it must also be granted that there are others who are strongly of opinion that *Trypanosoma dimorphon* and *Trypanosoma congolense* are distinct species. Under these circumstances it seems best to unite the old names under a new one, and *Trypanosoma pecorum* seems appropriate enough, as this trypanosome disease is peculiarly one of herds. Montgomery and Kinghorn have lately proposed the name *confusum,* in order to get out of the difficulty; but this name has been used for a trypanosome already, and it is, moreover, an awkward term.

It must then be understood that in the species *Trypanosoma pecorum* we include *Trypanosoma dimorphon, Trypanosoma congolense,* the trypanosome discovered in Zanzibar by Edington and described in the 'Proceedings,'* that from Portuguese East Africa and Zululand described by Theiler, and the species found in Northern Rhodesia by Montgomery and Kinghorn, and in Southern Rhodesia by Bevan.

The other species of trypanosomes found in the blood of cattle in Uganda were *Trypanosoma gambiense, Trypanosoma brucei, Trypanosoma vivax, Trypanosoma cazalboui,* and *Trypanosoma nanum.* These names, however, may require to be reconsidered.

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* B, vol. 81, p. 14.

DISTRIBUTION IN UGANDA.

Cattle suffering from *Trypanosoma pecorum* were sent to the Sleeping Sickness Commission's laboratory at Mpumu from Entebbe, Kampala, Namukekera, Kabula Muliro, and Mabira. It is probably widely distributed throughout Uganda. It was also found in a horse which had arrived in Nairobi, British East Africa, from Abyssinia. The Commission is indebted to Mr. Stordy, Principal Veterinary Officer, for the opportunity of studying this trypanosome. It is not known where the horse became infected, but it must have been at some point between Nairobi and the Abyssinian border.

MORPHOLOGY OF TRYPANOSOMA PECORUM.

A. *Living, Unstained.*

This trypanosome, when observed in a preparation of fresh blood, is seen to remain at or near the same spot in the field, that is to say, it is non-translatory. It is, however, active and restless, the body quivering rapidly, and the undulating membrane and flagellum keeping up a constant vibratory motion. As a rule, it moves with the flagellar end in front. The contents of the cell are homogeneous, except for a vacuole at the posterior extremity.

A marked characteristic of this species is that it exhibits alternating periods of quiescence and activity. When quiescent it is usually invisible, as it has a habit of burying itself under small collections of red blood corpuscles.

B. *Fixed and Stained.*

Method of Staining.—Giemsa's method, as described in a former paper,* was used.

Length.—The same method of measuring was used as described in the same paper (p. 17).

Breadth.—Without the undulating membrane the average is about 2 microns, with the membrane about 3 microns.

Shape.—This trypanosome when stained is short and stout in form. The posterior extremity is blunt, or rounded, or pointed and angular. The anterior end is narrower. The undulating membrane is fairly well developed, more so, perhaps, than in *Trypanosoma nanum.* The flagellum arises near the micronucleus, and passes along the edge of the undulating membrane. There is no free flagellum.

Contents of Cell.—Generally homogeneous. Sometimes granules are seen which take on a chromatin stain, and are situated anterior to the nucleus.

Nucleus.—Is oval in shape, and situated about the middle of the body.

---

* 'Roy. Soc. Proc.,' B, vol. 81, p. 16.

Table I.—*Trypanosoma pecorum.*

No. of experiment.	Animal.	Day of disease.	Method of fixing and staining.	In microns.		
				Average length.	Maximum length.	Minimum length.
82	Ox	—	Osmic vapour and Giemsa	11·7	14·0	10·0
505	,,	42	,,	12·3	15·0	10·0
	,,	—	,,	13·2	15·0	11·0
593	Sheep	50	,,	14·5	17·0	10·0
44	Monkey	31	,,	12·2	14·0	10·0
559	,,	14	,,	12·7	16·0	11·0
461	Dog	44	,,	15·3	18·0	13·0
543	,,	10	,,	14·3	16·0	11·0
1406	,,	31	,,	13·7	16·0	11·0
551	Rat	19	,,	12·6	18·0	8·0
626	,,	—	,,	12·8	15·0	10·0
398	Mouse	28	,,	14·6	16·0	13·0
398	,,	36	,,	13·1	17·0	10·0
			Average	13·3	16·0	10·6

Micronucleus.—Small and round, and situated near to, but not at, the posterior extremity.

Undulating Membrane.—Is simple, but fairly well developed.

Flagellum.—There is no free flagellum. In very rare cases, where there is an appearance of a free flagellum, the trypanosome will be found to be dividing.

As it is difficult to gain an idea of the general appearance of a trypanosome, a series of coloured drawings by Lady Bruce, R.R.C., is also given to supplement the written description (Plate 11). The trypanosomes have been stained by Giemsa's method, and drawn at a magnification of 2000. By referring to them, the shape, disposition of the micronucleus, and other parts of the structure of *Trypanosoma pecorum*, will be more readily understood.

As will be seen from this tabulated statement, the disease set up in domestic animals by *Trypanosoma pecorum* is a serious and fatal one.

Cattle, goats, sheep, monkeys, dogs, rats, and mice are susceptible. Guinea-pigs, on the other hand, are refractory. Horses, mules, donkeys, and rabbits were not available at Mpumu, so that, unfortunately, it is not possible to say whether they are inoculable or not. In regard to guinea-pigs it would be interesting to know whether a series of inoculations into rats or rabbits would make the *Trypanosoma pecorum* also pathogenic for them.

ANIMALS SUSCEPTIBLE TO TRYPANOSOMA PECORUM.

Table II.

No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Cattle.	
82	Natural infection	?	2	Died 2 days after arrival.
110	,,	?	243	Treated with lithium antimonyl tartrate.
230	,,	?	4	Died 4 days after arrival.
357	,,	?	3	,, 3 ,,
358	,,	?	1	,, 1 day after arrival.
359	,,	?	60	Treated with arsacetin.
360	,,	?	14	Died of *Trypanosoma pecorum.*
391	,,	?	313	,, ,,
475	,,	?	9	Died 9 days after arrival.
476	,,	?	2	,, 2 ,,
477	,,	?	13	,, 13 ,,
482	,,	?	5	,, 5 ,,
483	,,	?	34	,, 34 ,,
505	,,	?	265	Killed.
550	,,	?	—	Treated with arsenic; still alive after 254 days.
1459	,,	?	43	Died 43 days after arrival.
1560	,,	?	46	,, 46 ,,
1731	,,	?	79	Killed.
1733	,,	?	76	Died 76 days after arrival.
1735	,,	?	79	Killed.
1736	,,	?	79	,,
1737	,,	?	84	Died of *Trypanosoma pecorum.*
97	Ox	9	46	,, ,,
447	,,	8	287	Killed.
1463	,,	7	35	Died of *Trypanosoma pecorum.*
1464	,,	7	14	,, ,,
1225	Monkey	—	—	Died 8 days after inoculation; bitten by snake. Never showed trypanosomes.
1357	Dog	6	15	Died of *Trypanosoma pecorum.*
1358	,,	6	18	,, ,,
1359	,,	6	22	,, ,,
1363	,,	6	15	,, ,,
1364	,,	6	38	,, ,,
1365	,,	6	29	,, ,,
	Average......	6·7	63	
			Goat.	
1005	Ox	—	—	Never showed trypanosomes; still alive after 72 days.
1006	,,	14	44	Cause of death doubtful.
1404	,,	9	41	,, ,,
1405	,,	—	—	Never showed trypanosomes; under observation 31 days.
633	Monkey	12	51	Cause of death doubtful.
	Average......	11·6	45	

* Duration includes the days of incubation, it dates from the day of infection.

Table II—*continued.*

No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
			Sheep.	
697	Ox	21	—	Still alive after 170 days.
632	Monkey	8	43	Cause of death doubtful.
593	Dog	19	168	Died of *Trypanosoma pecorum.*
	Average......	16	105	
			Monkey.	
44	Ox	14	35	Died of *Trypanosoma pecorum.*
49	„	10	16	„ „
376	„	11	56	„ „
683	„	10	61	„ „
1740	„	11	—	Still alive after 45 days.
719	Sheep	21	—	„ 216 „
350	Monkey	10	181	Died of *Trypanosoma pecorum.*
459	„	6	80	„ „
1000	„	16	45	„ „
460	Dog	11	75	Killed for cultivation experiments.
559	„	12	39	Died of *Trypanosoma pecorum.*
560	„	14	32	„ „
581	Rat	13	86	„ „
	Average......	12·3	64	
			Dog.	
543	Horse	9	26	Died of *Trypanosoma pecorum.*
148	Ox	10	46	„ „
349	„	22	98	„ „
552	„	—	—	Re-injected after 17 days.
1007	„	—	—	Found dead after 14 days.
1406	„	21	29	Killed.
1407	„	—	—	Died; under observation 31 days.
1193	Monkey	8	16	Died of *Trypanosoma pecorum.*
433	Dog	7	44	„ „
434	„	9	58	„ „
461	„	6	40	„ „
1544	„	10	21	„ „
552	Rat	—	—	Still alive after 49 days.
	Average......	11·3	42	
			Guinea-pig.	
685	Ox	—	—	Still alive after 96 days.
1162	„	—	—	„ 36 „
1163	„	—	—	„ 36 „
1164	„	—	—	„ 36 „
1647	„	—	—	Died; under observation 29 days.
628	Monkey	—	—	Still alive after 104 days.
1002	„	—	—	„ 50 „
566	Dog	—	—	„ 78 „

* Duration includes the days of incubation, it dates from the day of infection.

Table II—*continued.*

No. of experiment.	Source of virus.	Period of incubation, in days.	Duration of disease, in days.*	Remarks.
		White rat.		
397	Ox	17	39	Died of *Trypanosoma pecorum.*
551	,,	16	18	Killed for cultivation experiments.
684	,,	16	32	Died of *Trypanosoma pecorum.*
699	,,	—	—	Experiment stopped after 57 days.
1646	,,	12	16	Died of *Trypanosoma pecorum.*
626	Monkey	12	22	Killed for cultivation experiments.
1001	,,	13	23	Died of *Trypanosoma pecorum.*
455	Rat	9	12	,, ,,
729	,,	11	21	,, ,,
1708	,,	8	12	,, ,,
	Average......	12·6	21	
		Mouse.		
686	Ox	—	—	Experiment stopped after 59 days.
398	Monkey	26	41	Died of *Trypanosoma pecorum.*
627	,,	12	26	,, ,,
454	Mouse	6	12	,, ,,
	Average......	14·7	26	

* Duration includes the days of incubation, it dates from the day of infection.

Theiler describes a trypanosome from Chai-Chai, near the mouth of the Limpopo, in Portuguese East Africa; from the mouth of the Zambesi; and also from Zululand, which resembles the one under discussion, in not infecting guinea-pigs; and considers that this one fact is sufficient for the creation of a new species. We cannot agree with him in this, as there is no practical importance, except in the laboratory, in the fact that the guinea-pig is insusceptible; and, moreover, until more experiments have been made, we cannot be sure that under certain conditions of dosage or passage through the smaller animals the guinea-pig will remain refractory.

The important facts in regard to this species are, that man is not susceptible, but that the valuable domestic animals are, and that in these animals the disease is, as a rule, a fatal one.

As long as our knowledge of trypanosomes is limited it seems better to group them under as few names as possible. As knowledge grows, and as fundamental differences emerge, then it will be time to define them more strictly. As far as our present knowledge goes, the morphology of

Trypanosoma dimorphon, *Trypanosoma congolense*, the Uganda, Zanzibar, Chai-Chai, Zambesi, Zululand, and Rhodesian strains is identical; these trypanosomes affect the same important domestic animals; the carrier is probably or may be the same, though this is not known, and therefore these various forms should, for the present, be grouped under one name; and for certain reasons given above we propose the name *Trypanosoma pecorum* for this group.

Disease set up in Cattle by Trypanosoma pecorum.

It is unnecessary in this paper to describe in detail the symptoms which can be noted during life, or the pathological changes set up in the organs of cattle by this trypanosome. It will be sufficient to say that the main symptoms are emaciation, anæmia, and progressive weakness, and that the principal *post-mortem* appearances are those due to anæmia.

Incubation.—As is probably true of most trypanosome diseases in susceptible animals, the period of time which elapses between the infection of the animal and the appearance of the trypanosomes in sufficient numbers in the peripheral blood to be seen by the microscope, is a short one: in this case, an average of 6·7 days.

Duration.—Of the course and duration of this disease in cattle little, unfortunately, is known. Most of the cattle which came under observation at the laboratory of the Commission at Mpumu were already sick when they arrived, and it was, as a rule, impossible to know when they had been infected. By referring to the table it will be seen that 22 naturally-infected cattle were under observation. Four of them lived, on an average, nine months. Of these four, one, treated with arsenic, was still alive and apparently healthy in December, 1909, one was killed, one had been treated with lithium antimonyl tartrate, and one died without treatment at the end of 313 days. It is therefore impossible to say from the insufficient data at our disposal whether spontaneous cure ever takes place in this disease in cattle.

When we turn to the cases of cattle which were inoculated on the hill, and were therefore under observation from the beginning, we are struck by the rapid course of the disease. One animal certainly lived 287 days, but the remainder died, on an average, in 26 days from the date of infection. Most of these oxen were inoculated with a strain of this trypanosome which had caused a rapidly fatal epidemic among a herd of milch cattle belonging to Mr. Walsh, at Kabula Muliro. In the short space of one month 24 of Mr. Walsh's cattle died, and in two months 34 had died out of a herd of about 300 head.

It may therefore be concluded that *Trypanosoma pecorum* sets up a rapid and fatal disease in cattle.

Disease set up in Goats and Sheep by Trypanosoma pecorum.

The number of cases of this disease in goats and sheep which came under observation is too small to draw any conclusions from. At Mpumu the goats and sheep were not satisfactory experimental animals, as many of them died from some unknown cause. It was thought that as these animals usually lived in the valleys, and were often housed in their owner's hut during the night, the exposure on the top of the hill had a bad influence. One sheep was still alive after 168 days, and it is probable that most of the goats and sheep would have lived much longer if they had been kept under more favourable conditions.

Disease set up in the Smaller Laboratory Animals.

It is not necessary to describe in detail the action of *Trypanosoma pecorum* on the monkey, dog, and smaller laboratory animals, as a reference to the table will show the average periods of incubation and duration. It will be seen that this is a fairly rapid and fatal disease in the dog, white rat, and mouse. In the monkey the average duration is about two and a-half months.

THE CARRIER OF TRYPANOSOMA PECORUM.

Glossinæ.—From experiments made in the laboratory at Mpumu it seems probable that *Glossina palpalis* is capable of acting as a carrier of this trypanosome. Four experiments were made with ordinary wild Lake-shore flies, and of these one was successful. Four were also made with laboratory-bred flies, and one again came off. The latter experiment, however, with laboratory-bred flies was not free from doubt; but from the other, which seemed free from doubt, it appears that *Trypanosoma pecorum* can develop in *Glossina palpalis* and infect a healthy animal after a period of 21 days. More observations are required. It may be noted that in no instance did *Trypanosoma pecorum* appear in the blood of animals upon which freshly-caught Lake-shore tsetse flies had been fed. These flies were found to be naturally infected with *Trypanosoma gambiense* and *Trypanosoma vivax.* This is an argument, though a small one, that *Glossina palpalis* is not the common or chief carrier of *Trypanosoma pecorum.*

Tabanidæ.—There is some circumstantial evidence available to show that *Trypanosoma pecorum* is carried by the *Tabanidæ.* In the valleys round Mpumu Hill, so far as we are aware, there are no tsetse flies at any time of the year. As a rule, there are a few *Tabanidæ.* The cattle belonging to the Commission went down to the foot of the hill every morning to graze, and returned to their kraal on the top at sunset. Half of the herd went to the east of the hill and half to the west. On both sides there was a small valley or glen, through which ran a small stream. In these valleys during the year, as a rule, a *Tabanus* or two or a *Hæmatopota* could be seen, but they were in small numbers. Now it is a curious fact that at certain times of the year enormous numbers of *Tabanidæ* will suddenly appear in places where only a few are, as a rule, to be found. For example, Mr. Brown, at Mabira, who was collecting the biting flies of his district, wrote on March 14, 1909, that the *Tabanidæ*, which for months had been scarce, were then swarming everywhere in countless numbers, and he afterwards wrote that this invasion lasted about a month. The particular species which appeared at Mabira at this time was *Tabanus socialis* (Walk.).

So, in the same way, at Mpumu, the *Tabanidæ*, which had been rare, suddenly appeared in swarms. They were first seen in the valley to the west of the hill in September, 1909, and a month later in the valley to the east. Soon after this the cattle which had shown no signs of disease during the previous year were found to be suffering from *Trypanosoma pecorum.* Those which grazed in the valley to the west were the first to be affected, and afterwards those which grazed to the east of the hill. The species of *Tabanidæ* in this case was *Tabanus secedens* (Walk.). In both groups of cattle there were cases of *Trypanosoma pecorum* disease, so that the *Tabanidæ* had a reservoir from which to draw the virus.

Another sudden epidemic of *Trypanosoma pecorum* disease occurred on Mr. Walsh's farm at Kabula Muliro, where, as stated above, 34 milch cattle died within two months in a herd of 300. The evidence is all against this epidemic having been caused by tsetse flies. During February and March, and again later in the year, during August and September, as many as 100 fly-boys were engaged scouring this district for biting flies. *Tabanidæ*, *Hæmatopota*, and *Chrysops* were brought in, but not a single tsetse, although a reward of 5 rupees was offered for each specimen. The commonest *Tabanus* in this district during August was *varictus* (Walk.).

It may, therefore, in our opinion, be concluded that the trypanosome disease caused by *Trypanosoma pecorum* can be carried from sick to healthy animals without the aid of *Glossinæ*, but what other species of fly, if any, acts as carrier is merely a matter of speculation at present.

Stomoxys.—Montgomery and Kinghorn state that they have strong evidence against this genus. At Mpumu several experiments were made to attempt to settle this question, but although they were persevered in for months, they remained negative. *Stomoxys* are so numerous in every part of the country all the year round that it seems inconceivable that they can act as carriers. From October, 1908, until the following September, although numerous cases of cattle with *Trypanosoma pecorum* in their blood grazed all day long with healthy cattle, yet not a single case of infection took place. The *Stomoxys* were exceedingly numerous all this time, forming a small cloud of flies round the cattle, and passing constantly from one animal to another, being driven hither and thither by the rapidly-swishing tails. This is a natural experiment on a large scale.

It will therefore require very convincing proof to bring this Commission to the belief that *Stomoxys* are carriers of this disease.

The subject of the carrier of *Trypanosoma pecorum* must remain in this unsatisfactory state for the present, but it is hoped that experiments which are at present being carried out at Mpumu may throw some light on this important part of the subject.

CULTIVATION OF TRYPANOSOMA PECORUM.

One difficulty experienced at Mpumu in attempts to cultivate the various trypanosomes was that rabbits were not available to supply the blood for Novy and MacNeal's medium. The blood of rats, goats, and oxen was used; but in making the cultivation of trypanosomes a factor in their diagnosis uniformity must be of the first importance.

Another difficulty was the frequency of contamination of the tubes. This was, perhaps, to be expected in a laboratory on the top of a hill in the tropics, with very free ventilation.

Trypanosoma pecorum grows fairly readily on blood-agar medium. At the end of 24 hours clumps may be seen containing many trypanosomes, with their flagellar ends directed outwards and in active motion (Plate 12, fig. 1). The individual flagellates have irregularly-shaped granules of chromatin scattered through their body substance, and also many brightly-refractile vacuoles. After 48 hours' culture every field contains many active trypanosomes, and also small clumps composed of 10, 20, or more members (Plate 12, fig. 2).

After six days the trypanosomes are still very active; they vary much in size and shape, from the plasmodial to the elongated, flagellated, highly active trypanosome. After this they seem to degenerate, and in a few days living trypanosomes can no longer be found in the tube.

This description approaches to some extent that of the cultural characters of Dr. Edington's trypanosome from Zanzibar and *Trypanosoma dimorphon*, but there is not that extraordinary growth which was described as covering several fields of the microscope. Whether this was due to the difference in the composition of the blood medium, or to the higher temperature at Mpumu, it is impossible at present to say.

By comparing the coloured plates of this trypanosome and the one from Zanzibar, a certain resemblance will be seen, but how far this should be taken as a factor in the diagnosis is difficult to say.

CONCLUSIONS.

1. *Trypanosoma pecorum* is an important trypanosome disease of domestic animals in Uganda.

2. It is similar in morphology, action on animals, and cultural characters, to the *Trypanosoma dimorphon* described by Laveran and Mesnil, and to Dr. Edington's trypanosome from Zanzibar, described in the 'Proceedings,'* except that *Trypanosoma pecorum* is not pathogenic to guinea-pigs.

3. The carrier is unknown, but is probably a *Tabanus*, and not *Stomoxys*.

DESCRIPTION OF PLATES.

PLATE 11.

This plate represents the general appearance of *Trypanosoma pecorum* in stained preparations. Note the rounded, blunt, or angular shape of the posterior extremity; the small, round micronucleus and vacuole placed near this end; the oval nucleus; and the absence of a free flagellum, except in dividing forms. Stained Giemsa, × 2000.

PLATE 12.

Fig. 1.—An aggregation, or clump, of *Trypanosoma pecorum* after 24 hours' growth in blood-agar. Stained Giemsa, × 2000.

Fig. 2.—The same after 48 hours. Stained Giemsa, × 2000.

Figs. 3, 4, and 5.—*Trypanosoma pecorum* after 3 days' growth. Stained Giemsa, × 2000.

Figs. 6 and 7.—*Trypanosoma pecorum* after 4 days' growth. Stained Giemsa, × 2000.

Figs. 8 and 9.—*Trypanosoma pecorum* after 8 days' growth. Stained Giemsa, × 2000.

Figs. 10, 11, and 12.—*Trypanosoma pecorum* after 10 days' growth. Stained Giemsa × 2000.

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* B, vol. 81, p. 14.

Experiments to ascertain if Cattle may act as a Reservoir of the Virus of Sleeping Sickness (Trypanosoma gambiense).

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(Received June 7,—Read June 16, 1910.)

The question as to whether cattle can act as a reservoir of the virus of Sleeping Sickness is an important one. It was usually believed until lately that man was the main reservoir, and that the other animals might be ignored. But in view of the fact that the fly on the Lake-shore have remained infective for some two years after the native population have been removed, it is necessary to enquire if it is not possible that other animals may act as well.

In this regard cattle have been, perhaps, the most important, as on the once thickly-populated Lake-shore and islands they were numerous, and in many cases grazed and watered in the fly-area. Another reason of their importance is, that if they can act as a reservoir, then the same will probably be true of the different species of antelope which inhabit the Lake-shore. It may be presumed that these will greatly increase now that the natives and domestic animals have been removed, and that they will take the place of the cattle in keeping up the infectivity of the *Glossina palpalis*.

The Commission, therefore, thought it would be well to enquire into the question, and the result is given in this paper.

Answers to the following questions were sought: Are cattle capable of being infected with Sleeping Sickness by the sub-cutaneous injection of blood containing *Trypanosoma gambiense?* Can cattle be infected with Sleeping Sickness by the bites of artificially-infected tsetse flies? Can cattle be infected with Sleeping Sickness by the bites of the naturally-infected flies caught on the Lake-shore? Is it possible to infect tsetse flies by feeding them on cattle infected with Sleeping Sickness, and afterwards to transmit the disease by means of these flies to healthy animals? Finally, if these questions are answered in the affirmative, will it be possible to find that cattle which have lived in the fly-area are naturally infected with Sleeping Sickness?

I. *Are Cattle capable of being Infected with Sleeping Sickness by the Subcutaneous Injection of Blood containing* Trypanosoma gambiense?

Experiment 869. Bull.

September 10, 1909.—A bull was inoculated with 5 c.c. of blood containing large numbers of *Trypanosoma gambiense* from an infected monkey.

Its blood was examined daily, and 18 days after injection the bull was found to be infected with *Trypanosoma gambiense.* The identity of the trypanosome was established by injecting a monkey with some blood from the ox. This monkey showed *Trypanosoma gambiense* on the sixth day.

Conclusion.—From this experiment it is seen that oxen are capable of being infected with Sleeping Sickness by the injection of blood containing *Trypanosoma gambiense.* The trypanosome appears in small numbers in the blood, and the blood, when injected into susceptible animals such as monkeys, gives rise to a fatal form of the disease.

II. *Can Cattle be Infected with Sleeping Sickness by the Bites of Artificially-infected* Glossina palpalis?

The two following experiments were carried out by feeding *Glossina palpalis* first on an infected monkey and immediately afterwards on a healthy ox. Wild flies from the Lake-shore were used.

Experiment 890. Ox.

May 20, 1909.—The ox was thrown and a monkey heavily infected with Sleeping Sickness was laid across its flank. Two cages of *Glossina palpalis,* containing 100 and 150 flies respectively, were allowed to feed for a few seconds on the monkey and then on the ox. The flies were allowed from 30 to 35 interrupted feeds on each animal every day. This was continued for 38 days, during which time 561 flies were estimated to have fed on one or other animal.

July 17.—Fifty-eight days after the first infected feed, *Trypanosoma gambiense* appeared in the blood of the ox.

The identity of the trypanosome was established by injection of the ox's blood into two monkeys. The first monkey was injected with blood from the ox 76 days and the second monkey 181 days after the flies had first fed on the ox. Both monkeys developed Sleeping Sickness, the first 7 days and the second 11 days after injection of the blood.

Experiment 891. Calf.

The details of this experiment were similar to those of the last. *Trypanosoma gambiense* appeared in the blood of the calf 57 days after the flies had been first fed upon it.

Three cubic centimetres of the blood of the calf were injected into a monkey, and the monkey developed Sleeping Sickness after an incubation period of 8 days.

Conclusion.—These two experiments show that when artificially infected *Glossina palpalis* are allowed to feed on healthy cattle, these animals develop Sleeping Sickness, and that the blood of the cattle is capable of giving rise to infection of *Trypanosoma gambiense* in monkeys when injected into them.

III. *Can Cattle be Infected with Sleeping Sickness by the Bites of the Naturally-infected Flies caught on the Lake-shore?*

In the next three experiments freshly-caught *Glossina palpalis* brought up to the laboratory from the Lake-shore were allowed to feed straightway on healthy cattle. By this means it will be shown whether *Glossina palpalis* in their wild state are capable of giving Sleeping Sickness to healthy cattle.

Experiment 982. Bull.

2195 freshly captured *Glossina palpalis* were applied to a bull, and of these 1536 were estimated to have fed. This feeding of the flies extended over a period of 16 days, at the end of which time *Trypanosoma gambiense* appeared in the blood of the bull.

To help in the identification of this trypanosome, 3 c.c. of the blood of the bull were injected into a monkey. The monkey developed Sleeping Sickness 18 days later. 5 c.c. of the blood of the bull were also injected into a goat. *Trypanosoma gambiense* appeared in the blood of the goat after an incubation period of 38 days.

Experiment 1462. Bull.

The details of this experiment were similar to those of the last one. Over a period of eight days, 1370 wild flies from the Lake-shore were applied to the bull, of which 705 fed. Ten days from the first application of flies, *Trypanosoma gambiense* appeared in the blood of the bull.

Two animals, a monkey and a goat, each received 1 c.c. of the blood of the bull by injection under their skin. The monkey developed Sleeping Sickness seven days later, but the goat died in 16 days without showing any infection.

Experiment 1465. Bull.

During a period of 13 days, 459 freshly caught Lake-shore *Glossina palpalis* were applied to a bull, and of these 314 fed. On the fourteenth day after the flies were first fed, the bull developed an infection of *Trypanosoma gambiense.*

Some blood from this bull was injected into a monkey and into a goat. Neither animal became infected.

Conclusion.—These experiments prove that *Glossina palpalis*, when captured in their natural state on the Lake-shore, are capable of transmitting the virus of Sleeping Sickness to cattle, and that the blood of these cattle gives rise to a fatal form of the disease in monkeys and in goats when it is injected into them.

IV. *Is it possible to Infect Tsetse Flies by Feeding them on Cattle Infected with Sleeping Sickness, and afterwards to transmit the Disease by means of these Flies to Healthy Animals?*

Five experiments under this heading were carried out. Laboratory-bred flies were used in all of them. Three were negative and two positive. The three negative experiments will be shortly summarised first.

Experiment 1451.

Ninety laboratory-bred *Glossina palpalis* were fed for 10 successive days on a calf whose blood contained *Trypanosoma gambiense.* The flies were starved for 72 hours. They were then fed on a clean monkey daily for 45 successive days. The monkey failed to develop Sleeping Sickness.

When the remainder of the flies were dissected, one contained flagellates, but when the contents of this fly were injected into a goat the animal failed to show any infection of *Trypanosoma gambiense.*

Result.—Negative.

Experiment 1269.

The details of this experiment were similar to those of the last. After the *Glossina palpalis* had been fed on two oxen whose blood contained *Trypanosoma gambiense*, they were applied daily to a monkey. They were fed on this monkey for 35 consecutive days and were then transferred to a second monkey. Both the monkeys remained healthy.

Two of the flies were found on dissection to contain flagellates, but when these were injected into a monkey and a goat no development of Sleeping Sickness took place in these animals.

Result.—Negative.

Experiment 1672.

Here again the technique was similar to the last. The *Glossina palpalis* were fed, on alternate days for a lengthened period, on a clean monkey and a clean goat. Both animals remained healthy.

Some infected flies were found on dissection, but when introduced under the skin of a goat and of a monkey did not give rise to Sleeping Sickness.

Result.—Negative.

The next two experiments, which were carried out in the same way as the two preceding ones, were positive.

Experiment 1566.

The *Glossina palpalis* were fed on an infected ox, and after a starve of 72 hours were fed on a clean monkey for 45 successive days. 68 days after the flies had taken their first infected feed, this monkey developed Sleeping Sickness.

When the flies came to be dissected, nine of them showed flagellates either in the proboscis or in the alimentary tract. Some of these were injected into goats and into a monkey, but with negative results.

Result.—Positive.

Experiment 1602.

Fifty laboratory-bred flies were fed for four successive days on an ox whose blood contained *Trypanosoma gambiense.* After a period of starvation they were applied to a monkey and to a goat on alternate days. The monkey died before it could have become infected, but the goat developed Sleeping Sickness 20 days after the flies had their first infected feed.

The remainder of the flies, 32 in all, were dissected, and five were found to contain flagellates. The alimentary contents of one of these flies were injected into a monkey, and after an incubation period of 13 days *Trypanosoma gambiense* appeared in its blood.

Result.—Positive.

Conclusion.—Laboratory-bred tsetse flies can be infected by feeding them on cattle infected with Sleeping Sickness, and afterwards the disease can be transmitted to healthy animals by means of these flies.

V. *Do Cattle, when Living in the Fly-area, actually carry the Virus of Sleeping Sickness?*

About seventeen cattle from various sources were examined with this point in view. Not all these cattle could be proved to have been exposed to the bites of *Glossina palpalis*, but most of them came from places where these flies are plentiful. One was positive.

Experiment 1633.

This cow came from the island of Kome, in Lake Victoria, where human Sleeping Sickness is prevalent and where *Glossina palpalis* abound.

Trypanosoma gambiense was found in its blood by microscopical examination, and when 3 c.c. of the blood were injected under the skin of a monkey the animal developed Sleeping Sickness after an incubation period of seven days.

Conclusion.—This experiment proves that cattle in their natural state, and apparently in good health, may harbour the virus of Sleeping Sickness.

General Conclusions.

It has been proved by experiment that cattle may act as a reservoir of the virus of Sleeping Sickness, and that healthy animals may be infected from them by means of *Glossina palpalis.*

It has also been proved that cattle in the fly-area do naturally harbour *Trypanosoma gambiense.*

It is, therefore, possible that the cattle and antelope living in the fly-area may act as a reservoir, and so keep up the infectivity of the *Glossina palpalis* for an indefinite period, but there is no proof up to the present that this actually takes place in Nature.

"*Muhinyo*," *a Disease of Natives in Uganda.*

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(Received June 7,—Read June 30, 1910.)

When the Sleeping Sickness Commission passed through Kampala, the native capital of Uganda, at the end of October, 1908, on their way to their camp at Mpumu, they were informed by Sir Apolo Kagwa, K.C.M.G., the Prime Minister, that a new disease had broken out in the province of Ankole, and that many people were sick.

This is probably the same disease which was described by Dr. A. G. Bagshawe in 1906. He gives the history and symptoms of nine cases which he saw in Ankole. He concluded that the disease was beri-beri, and states that at one village 25 per cent. of the inhabitants were suffering from a more or less severe form of the disease.

In the same year Dr. L. D. Lowsley also described "Muhinyo," but was of opinion that it might possibly be dengue with persistent joint pains.

Nothing more seems to have been written about "Muhinyo" until the beginning of 1909, when Dr. A. C. Rendle reported its presence in large numbers in the country round Lake Albert Edward. He says that all classes suffer, and that he has no hesitation in saying that "the disease is closely allied to kala-azar, the black death of India."

Thanks to the kindness of Sir Apolo Kagwa and Chief Saulo Mayanja Lumama, the Commission had an opportunity of seeing a case of "Muhinyo," which was sent to Mpumu from Ankole in January, 1909. This patient, who was said to have been ill for three months, was extremely weak and thin, but otherwise he showed no symptoms which pointed to any special disease.

As no other cases could be sent such a long journey as to Mpumu, it was decided that a member of the Commission should proceed to the district, in order to examine sick natives whom A. H. Watson, Esq., the District Commissioner, had kindly undertaken to have collected there.

On May 23, 1909, Dr. A. D. P. Hodges, the Principal Medical Officer, Uganda Protectorate, accompanied by Colonel Sir David Bruce, Director of the Commission, went to Masaka on the borders of Ankole, where they found some 50 sufferers from this disease awaiting them.

Distribution of "Muhinyo" in Uganda.

The principal focus of the disease is along the eastern shore of Lake Albert Edward, which corresponds nearly to longitude 30° E., in the latitude of the Equator. The most severe cases have been met with at Katwe (Fort George), a settlement on the eastern shore of Lake Albert Edward. It appears to have spread down the eastern shore of this lake, and to have extended in a south-easterly direction into Ankole. Cases have been recorded as far east as the western shore of Lake Victoria, and as far north as the Katonga River, which runs parallel to, and about 10 miles north of, the Equator. The disease is therefore quite limited in its distribution.

There is no evidence to show how it originated.

Epidemiology.

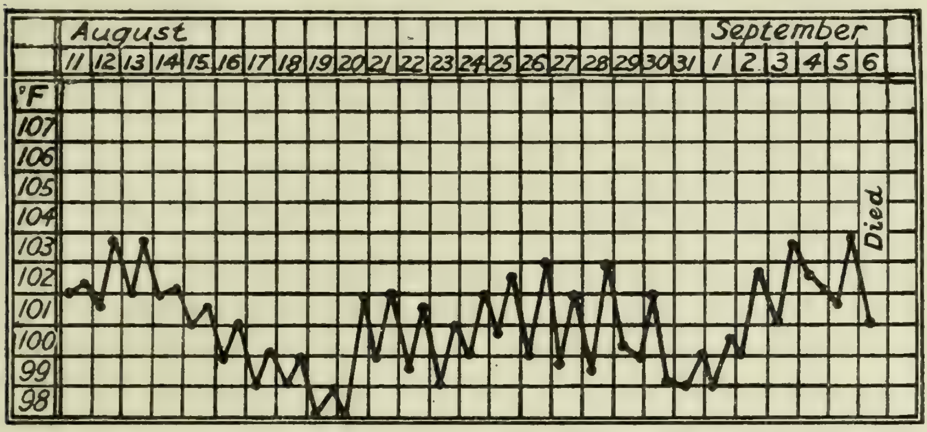
The tribes most effected by the disease are the Bakonjo and the Basongora. The former are morally and socially about the lowest class of people to be met with in Uganda. They are abjectly poor and dirty in their persons and in their habits. They live in rude grass huts, which they share with their domestic animals. The Bakonjo keep goats and, if they can afford them, cattle also. They prefer the milk of the cow, but also drink largely of goats' milk. The Basongora are a higher type of native, and resemble in appearance and customs the Bahima, the aristocracy of Uganda. They are not so poor as the Bakonjo, whom they use as serfs; they keep cattle and goats, and consume the milk of both animals. The flesh of the goat is largely eaten by both classes in a partially cooked state. The milk of sheep is occasionally used in default of that of the other animals.

Clinical Symptoms of "Muhinyo."

As the result of the examination of the 50 cases sent into Masaka, it appeared that the principal symptoms of "Muhinyo" are fever, profuse sweating, pains in the joints and along the course of nerves, swelling of the various joints, especially the ankles, and extreme weakness and emaciation. The disease is of long duration; most of the patients had been ill for several months. In 13 cases, taken at random, the average duration was three and a-half months. Another had been ill for two years.

The following temperature chart was the only one which could be obtained.

In most of the cases there was no marked enlargement of the spleen or liver, nor symptoms of paresis or paralysis. The microscopical examination of the blood showed various degrees of anæmia, but no parasites or marked changes in the white blood corpuscles could be detected. Further, the examination of the splenic pulp, obtained by puncture of the spleen, failed to reveal the presence of the parasites of kala-azar. It was therefore evident that "Muhinyo" was neither kala-azar nor beri-beri, but the long duration of the fever, the joint pains, and the extreme weakness and emaciation suggested a continued fever, such as typhoid or Malta fever.



Examination of the Blood for Agglutinative Phenomena.

The blood of several of these cases was therefore tested with *Bacillus typhosus* and *Micrococcus melitensis* by Widal's method, with the result that no reaction was obtained with the former, but positive results, in fairly high dilutions, were got with the latter.

The following table represents the result of the examination of the blood of "Muhinyo" with a strain of *Micrococcus melitensis* from Malta, from which it will be seen that five out of the seven cases examined gave a positive reaction:—

Table I.

No. of experiment.	Dilution of serum.			Control.
	1 in 50.	1 in 100.	1 in 200.	
927	+	+	−	−
928	+	+	−	−
929	−	−	−	−
930	−	−	−	−
931	+	+	+	−
932	+	+	+	−
933	+	+	−	−

Isolation of the Micrococcus of Malta Fever from the Spleens of Cases of "Muhinyo."

The next thing to be undertaken was the isolation of the *Micrococcus melitensis* from the tissues of patients suffering from "Muhinyo."

The spleens of two cases (925 and 926) were punctured in the usual way, and the splenic pulp smeared on the surface of tubes of nutrient agar-jelly. Small white colonies were grown from both cases, and these were subcultured and used to study the morphology, cultural characters, and animal reactions of the organism of which they were composed.

Morphology.—Under the microscope the organisms were found to be minute micrococci, indistinguishable in size or appearance from the *Micrococcus melitensis.*

Cultural Characters.—In the same way it was found that the sub-cultures of the organism showed after some days as minute transparent colonies, resembling tiny drops of dew, which afterwards became more opaque, and in no way differed from colonies of *Micrococcus melitensis* cultivated under the same circumstances.

Animal Reactions.—The sub-cultures were also emulsified in saline solution and injected into a monkey and rabbit. The monkey sickened with fever, and when the agglutinating power of its blood was tested with the strain of *Micrococcus melitensis* from Malta it was found to give a complete reaction in a dilution of 1 in 200. Having thus proved that two animals treated with the "Muhinyo" organism gave a serum capable of agglutinating a known *Micrococcus melitensis* from Malta, the converse experiment was made.

A rabbit was inoculated with the Malta strain, and its serum tested on the "Muhinyo" organism. This rabbit's serum, immunised against Malta fever, agglutinated the "Muhinyo" organism in a dilution of 1 in 200; and thus the proof that the micrococcus obtained from the spleen of "Muhinyo" cases and that obtained from cases of Malta fever were identical, was established.

Examination of Goats from the "Muhinyo" District, to ascertain if they are reservoirs of the virus of Malta Fever.

By Widal's Reaction.—In Malta, the Royal Society Commission discovered, in 1905, that the drinking of goats' milk was the sole mode of infection in Malta fever. Many of the Maltese goats were examined, and 50 per cent. of them found to be affected in some way by the disease, while 10 per cent. were actually excreting the *Micrococcus melitensis* in their milk.

It was, therefore, a matter of importance, as well as curiosity, to ascertain if the Ankole goats also suffered from Malta fever, and if the causation of this disease was the same in Central Africa as it had been proved to be on the shores of the Mediterranean, in the Soudan, and in South Africa.

When Sir Apolo Kagwa was approached as to the feasibility of obtaining goats from the most affected districts, he informed the Commission that he would see what could be done. About six weeks later a flock of goats, numbering in all 24, was driven up to the laboratory at Mpumu, and it was stated that these had come from a place where "Muhinyo" was common. They were at once examined, with the result that the blood of three out of their number reacted to the strain of *Micrococcus melitensis* obtained from cases of "Muhinyo," and also to the Malta strain.

The following tables give the details :—

Table II.—*Micrococcus melitensis* ("Muhinyo" strain).

No. of experiment.	Dilutions of serum.					Control.
	1 in 10.	1 in 20.	1 in 50.	1 in 100.	1 in 200.	
1512	+	+	+	−	...	−
1507	+	+	+	+	...	−
1776	+	+	+	+	+	−

Table III.—*Micrococcus melitensis* (Malta strain).

No. of experiment.	Dilutions of serum.				Control.
	1 in 10.	1 in 20.	1 in 50.	1 in 100.	
1512	+	+	+	−	−
1507	+	+	+	+	−

Isolation of the Micrococcus of Malta Fever from the Tissues of the Goats.—After having found that some of the Ankole goats reacted to the agglutination test, an attempt was made to isolate the *Micrococcus melitensis* from their tissues. This proved successful in two cases. The following experiment gives one of these in detail :—

Experiment 1475. Goat.

August 11, 1909.—This goat, which was one of a herd from Ankole, died this morning. The spleen was removed, and small portions of the pulp spread over the surface of agar tubes.

August 16.—A growth consisting of several very small, round, white colonies appeared after three days. A stained preparation from one of these showed that they were composed of organisms resembling *Micrococcus melitensis*. Sub-cultures made.

September 29.—The growth from two agar tubes was made into an emulsion with salt solution, and an agglutination test made with serum from a rabbit immunised against *Micrococcus melitensis*, Malta strain.

The result was that the organism from the goats agglutinated completely in a dilution of 1 in 100, and the proof was complete that the Ankole goats are liable to contract Malta fever, and to act as a reservoir of the virus.

Conclusions.

1. "Muhinyo" is Malta fever.
2. "Muhinyo" is conveyed from the goat to man by the drinking of goats' milk.

The Natural Food of Glossina palpalis.

By Colonel Sir David Bruce, C.B., F.R.S., Army Medical Service; Captains A. E. Hamerton, D.S.O., and H. R. Bateman, Royal Army Medical Corps; and Captain F. P. Mackie, Indian Medical Service. (Sleeping Sickness Commission of the Royal Society, Uganda, 1908–10.)

(Received June 15,—Read June 30, 1910.)

As a good deal of interest, and it may be importance, attaches to the food of the tsetse fly—the carrier of Sleeping Sickness—the following notes are placed on record:—

In the laboratory it was found that the flies fed with far more avidity on birds than on monkeys, while they could hardly be tempted to feed on young crocodiles, iguanas, or lizards. It was very marked, this preference of theirs for birds; the moment a chicken was placed against the netting of the cage, they instantly swarmed on it in hundreds. From this it was thought probable that the natural food of the flies would prove to be birds' blood, but the two following experiments do not bear this out to any great extent:—

The first experiment was carried out in the laboratory at Mpumu, and extended over several months. Flies which had been caught on the Lake-shore and which had been kept over from the previous day, and sometimes longer, were dissected, and each portion of the alimentary canal examined in the fresh state under a low power. The various organs of the fly were then smeared separately, and after staining (with one of the modifications of the Romanowsky stain) were examined in the usual way.

The dissections were carried out as follows:—The pharynx and proboscis were removed with a needle, and having been placed under a cover-glass and slightly opened out by pressure were examined in the fresh state. The tube of the proboscis was in this way admirably displayed, and the presence of red blood corpuscles or of flagellates could readily be determined. The alimentary canal was then removed from the abdomen, and the salivary glands detached from the fat-body. The alimentary canal was then unravelled and laid out in its full length on a slide. The whole of the tube could then be passed in review, from the junction of the thoracic intestine with the fore-gut down to the rectum, and the contents at various levels determined, as far as possible, with medium power magnifications. The main portions of the alimentary canal were then noted, and these were separated from one another by cross cuts with a sharp-edged dissecting needle. In this way the fore-gut, mid-gut, hind-gut, and proctodæum were separately removed and smeared on a slide. The smears (whilst still moist) were exposed to the vapour of osmic acid for a few seconds, and then passed through alcohol before washing and staining. The proventriculus can, with a little practice, be removed intact from the ventral aspect of the thorax. If necessary, the thoracic portions of the salivary ducts can also be recovered.

Speaking in general terms, the contents of the various portions of the alimentary canal could be determined with considerable accuracy from the naked-eye appearance during dissection. If the fly has fed very recently, the blood oozes from its proboscis when it is handled. The proventriculus, the crop or sucking stomach, as well as the fore-gut, are greatly distended with red blood. When the fed fly is put aside the blood-cells generally disappear from the proboscis in a few hours, though they may exceptionally be found as long as 24 hours after a feed. Similarly, the proventriculus and thoracic intestine quickly empty themselves, and the crop discharges its contents into the upper alimentary canal within 24 hours.

The blood travels slowly down the alimentary canal, changing in appearance as it goes. The outline of the red cells can sometimes be distinguished three days after a feed, but only when they have been retained in the fore-gut. When the blood reaches the mid-gut it begins to disintegrate and becomes homogeneous and somewhat translucent; when it reaches the lower part of the mid-gut it first becomes dark and eventually black and tarry. When in this state all cell elements are lost, and the colouring matter of the red cells is recognisable in the form of amorphous black granules. When the contents pass posterior to the Malpighian tubes, that is, into the hind-gut, they at once become fæcal in character, and turn into a dirty, yellowish-brown material, which is microscopically composed of fine granules. It is passed from the fly in this form.

The figures given in the subjoined table refer to flies which were caught at various parts of the Lake-shore, and were generally kept for about 24 hours before dissection. The majority of the dissections were done during October, November, and December, 1908.

Table I.—The Contents of the Alimentary Canal of 220 *Glossinæ palpalis.*

Total flies examined.	Intestine empty.	Intestine contained blood.	Character of Blood.		
			Mammalian.	Non-mammalian.	Non-recognisable.
220	160	60	20	9	31
Percentages...	72·7	27·3	9·1	4·1	14·1
			27·3		

Remarks.—In two cases blood corpuscles were recognised as being derived from monkeys, as the characteristic parasites of monkey malaria were found in them.

The second experiment to ascertain the food supply of *Glossina palpalis* was modified as follows:—

A journey was made to a small peninsula, hereafter referred to as "Crocodile Point." This place was distant about two and a-half to three hours by canoe from the Kibanga landing-place, and lay in a sheltered bay far out of the beaten track of the canoes which come backwards and forwards between the islands of Kome, Damba, and Buvuma, and the weekly market at Kibanga clearing. It was chosen, therefore, partly because it was isolated from human influences, and also because of the large number of flies which lived there and the number of crocodiles and birds which frequented it.

When it was first visited, a large crocodile was disturbed from where she was lying outstretched on a spit of sand. The canoe-men at once set to work, and unearthed 58 crocodile eggs lying in layers about 18 inches below the surface, over which the "form" of the parent was clearly defined in the soil.

The peninsula was pointed in shape, and not more than 60 yards long, and was closed on the land side by the dense wall of forest which fringes the Lake-shore. It was scattered with light undergrowth and fringed by ambatch trees, on which flocks of divers and cormorants sat with outstretched wings drying in the sun. A barrier of bare rocks and boulders projected on one side, and a small school of hippopotami was generally to be seen floating near and basking in the sun. Many small crocodiles were disturbed from the undergrowth as the point was explored, and various kinds of small land birds frequented the reeds and shrubs. The soil was sandy loam, and shaded by the light foliage. *Glossina palpalis* swarmed. The place has been described at some length, so that the exact conditions may be realised.

Subsequent to this experiment, the daily catch of Lake-shore flies was obtained from this place, and incidentally it may be added that the flies caught here were regularly found to be infective to monkeys.

Table II.—The Naked-Eye Appearances of the Contents of the Alimentary Canal of 183 Tsetse Flies, with their Sex, and the Presence or Absence of a Larva.

No.	Sex.	Larva.	Red blood.	Black blood.	No.	Sex.	Larva.	Red blood.	Black blood.
1	♂	−	−	−	34	♂	−	−	−
2	♂	−	−	−	35	♀	−	+	−
3	♂	−	−	−	36	♀	−	−	+
4	♀	+	−	−	37	♀	+	−	−
5	♀	−	−	−	38	♂	−	+	−
6	♂	−	−	−	39	♀	−	−	+
7	♂	−	−	−	40	♀	−	−	+
8	♀	+	−	−	41	♂	−	−	+
9	♂	−	−	−	42	♂	−	+	−
10	♀	−	−	−	43	♂	−	−	+
11	♀	−	−	−	44	♀	+	−	−
12	♀	−	−	−	45	♀	−	−	+
13	♀	−	−	−	46	♂	−	−	+
14	♂	−	−	−	47	♀	−	−	+
15	♂	−	−	−	48	♀	−	−	+
16	♂	−	−	−	49	♂	−	−	+
17	♀	−	−	+	50	♂	−	−	−
18	♀	−	−	+	51	♂	−	−	+
19	♂	−	+	−	52	♀	+	−	−
20	♀	−	−	+	53	♂	−	−	−
21	♂	−	+	−	54	♀	−	+	+
22	♂	−	−	+	55	♂	−	−	−
23	♀	−	−	+	56	♀	−	−	+
24	♀	−	−	+	57	♂	−	−	−
25	♂	−	+	−	58	♀	+	−	−
26	♂	−	−	+	59	♀	−	−	+
27	♂	−	+	+	60	♀	−	−	+
28	♀	+	−	+	61	♂	−	−	+
29	♂	−	−	+	62	♂	−	−	+
30	♂	−	−	−	63	♂	−	−	+
31	♀	−	−	+	64	♀	−	−	−
32	♀	+	+	−	65	♂	−	−	−
33	♀	−	−	+	66	♂	−	−	+

Table II—*continued.*

No.	Sex.	Larva.	Red blood.	Black blood.	No.	Sex.	Larva.	Red blood.	Black blood.
67	♂	–	–	–	126	♂	–	–	+
68	♂	–	–	–	127	♀	–	–	–
69	♂	–	–	–	128	♂	–	–	+
70	♂	–	–	–	129	♀	–	–	+
71	♀	–	+	–	130	♀	–	–	+
72	♂	–	–	+	131	♂	–	+	+
73	♀	–	–	–	132	♂	–	+	+
74	♀	+	+	+	133	♂	–	+	+
75	♂	–	+	+	134	♀	–	+	+
76	♂	–	–	+	135	♂	–	–	–
77	♂	–	+	+	136	♂	–	–	–
78	♀	–	+	–	137	♀	+	–	+
79	♂	–	–	–	138	♀	–	–	+
80	♂	–	–	–	139	♂	–	–	–
81	♀	–	–	–	140	♂	–	–	+
82	♂	–	+	+	141	♂	–	–	+
83	♀	+	–	–	142	♂	–	–	+
84	♀	+	–	–	143	♂	–	+	+
85	♂	–	–	–	144	♂	–	–	–
86	♀	–	–	+	145	♂	–	–	+
87	♂	–	–	–	146	♂	–	–	–
88	♀	–	–	–	147	♂	–	+	+
89	♂	–	–	–	148	♀	+	–	+
90	♂	–	–	+	149	♀	+	–	+
91	♀	+	+	+	150	♀	–	–	+
92	♀	+	–	–	151	♀	–	–	+
93	♂	–	–	–	152	♀	–	–	+
94	♂	–	–	+	153	♀	+	–	+
95	♂	–	–	+	154	♂	–	–	+
96	♂	–	–	+	155	♂	–	–	–
97	♂	–	+	+	156	♂	–	–	–
98	♀	–	+	+	157	♂	–	+	+
99	♂	–	–	–	158	♂	–	–	–
100	♂	–	–	+	159	♀	–	–	+
101	♀	+	–	+	160	♂	–	–	–
102	♀	+	–	+	161	♀	+	–	+
103	♂	–	–	–	162	♂	–	–	–
104	♀	+	–	–	163	♂	–	–	+
105	♂	–	–	–	164	♂	–	–	–
106	♀	+	–	+	165	♂	–	–	–
107	♂	–	–	–	166	♂	–	–	–
108	♂	–	–	+	167	♀	+	–	+
109	♂	–	–	–	168	♀	+	–	+
110	♂	–	–	–	169	♀	–	+	–
111	♀	+	–	–	170	♀	+	–	+
112	♂	–	–	+	171	♂	–	–	+
113	♀	–	–	+	172	♂	–	–	+
114	♂	–	–	+	173	♀	+	–	+
115	♂	–	–	–	174	♂	–	–	–
116	♂	–	+	+	175	♂	–	–	+
117	♀	–	–	+	176	♂	–	+	+
118	♂	–	–	–	177	♀	+	+	+
119	♀	+	–	+	178	♀	+	–	–
120	♂	–	+	+	179	♀	+	–	–
121	♀	–	+	+	180	♀	+	–	+
122	♀	–	–	–	181	♀	–	–	+
123	♀	–	+	+	182	♂	–	–	–
124	♂	–	+	+	183	♂	–	–	–
125	♂	–	–	–					

In the present experiment, the observer landed by canoe and made the paddlers sit round him; the fly-boys moved about amongst them and caught the flies as they came to feed. They were handed at once to the observer, who snipped off their heads, noted their sex, and roughly dissected out the alimentary canal, and, when it contained food-stuff, smeared it at full length on a slide. Notes were made as to the naked-eye contents of the canal and the question of pregnancy. The smears were fixed in the usual way and brought to the laboratory, where they were stained and examined minutely.

The total number of flies examined was 183, of which 104 (57 per cent.) were males and 79 (43 per cent.) were females; of the total number, 108 (59 per cent.) contained blood in a more or less digested state, and 75 (41 per cent.) contained no food-stuff. Out of the 79 females, 32 (40 per cent.) contained nearly fully-developed larvæ.

Table III.—Shows the Nature of the Blood in the Interior of the Flies, and also the Number of Flies which contained Parasites.

No.	Mammalian blood.	Non-mammalian blood.	Trypanosomes.	Halteridia.	No.	Mammalian blood.	Non-mammalian blood.	Trypanosomes.	Halteridia.
1	–	+	–	–	27	–	+	–	–
2	–	+	–	+	28	–	+	–	–
3	–	+	+	–	29	–	+	–	–
4	–	+	–	–	30	–	+	+	–
5	–	+	–	–	31	–	+	–	–
6	–	+	–	–	32	+	–	–	–
7	–	+	–	–	33	–	+	+	–
8	–	+	–	–	34	–	+	–	–
9	–	+	–	–	35	–	+	–	–
10	–	–	+	–	36	–	–	+	–
11	+	–	–	–	37	–	+	–	–
12	–	+	–	–	38	–	–	+	–
13	–	–	+	–	39	–	+	–	–
14	–	+	–	+	40	+	–	–	–
15	–	–	+	–	41	–	–	+	–
16	–	+	–	–	42	–	+	–	–
17	–	+	–	+	43	–	+	–	+
18	–	–	+	–	44	–	+	–	–
19	–	+	–	–	45	–	+	–	–
20	–	–	+	–	46	+	–	–	–
21	+	+	–	–	47	–	+	–	–
22	–	+	–	–	48	–	–	+	–
23	–	+	–	–	49	–	–	+	–
24	–	+	Trypanoplasma	–	50	–	+	–	–
					51	+	–	–	–
25	–	+	–	+	52	+	–	–	–
26	–	–	+	–					

The above table shows that out of the original 183 flies examined, 52 (28 per cent.) contained blood which was recent enough to show the red corpuscles. Out of these 52, 7 (13 per cent.) were from mammalian blood, whilst non-mammalian blood was present in 35 flies (67 per cent.). Trypanosomes were present in 14 flies (27 per cent.) and Halteridia in 5 (nearly 10 per cent.). One contained a trypanoplasma, derived, probably, from bird's blood.

An attempt was made to separate the nucleated red blood corpuscles into avian and reptilian. The distinction was made by size only, it being found from the measurement of corpuscles from birds and reptiles that the average normal length was:—

Standard amphibian (crocodile), 15·4 microns.
Standard avian (horn-bill), 13·1 microns.

In examining the smears from the flies, the average was taken of 10 to 20 red cells, which seemed as natural as possible, and the average obtained in

Table IV.—Shows the Average Measurements of the Nucleated Red Corpuscles and their Probable Origin.

No.	Average length of red cells, in microns.	Probably reptilian or amphibian.	Probably avian.
1	15·3	+	—
2	14·2	+	—
3	14·1	+	—
4	13·3	—	+
5	14·4	+	—
6	14·0	+	—
7	14·0	+	—
8	14·4	+	—
9	14·5	+	—
10	13·0	—	+
11	14·8	+	—
12	14·4	+	—
13	10·6	—	+
14	13·4	—	+
15	14·6	+	—
16	13·2	—	+
17	14·0	+	—
18	15·2	+	—
19	13·2	—	+
20	13·3	—	+
	Totals.........	13	7

Remarks.—Only 20 of the flies contained nucleated blood which was recent enough to justify any deduction as to its origin. Out of these 20 flies, 13 had probably fed on a reptile or amphibian, and 7 on a bird.

this way compared with the standard measurements. No allowance could be made for alterations due to digestive changes: these were presumed to be similar in all cases.

Table V.—Showing the Result of the Examination of *Glossina palpalis* from "Crocodile Point."

No. of flies.	♂.	♀.	♀ containing larvæ.	Intestine empty.	Intestine containing blood.	Mammalian blood.	Non-mammalian blood.	Non-mammalian blood.		Flagellates.	Halteridia.
								Avian.	Reptilian.		
183	104	79	32	75	108	7	35	7	13	14	5
Percentages...	57	43	17·4	41	59	3·8	19·1	3·8	7	7·6	2·7

Conclusions.

Two hundred and twenty *Glossinæ palpalis* were caught on various parts of the Lake-shore, and at intervals extending over several months; they were examined about 24 hours after capture. The examination of their intestinal contents revealed the fact that about 27 per cent. contained the remains of blood, the majority of which was of mammalian origin.

In the second experiment, 183 *Glossinæ palpalis* were caught at one spot where the food supply was abundant—birds and crocodiles—and the flies were examined at once. A much higher percentage (nearly 60 per cent.) contained the remains of a blood meal. The blood in the majority of the flies had been obtained from birds or reptiles, and of these the reptilian blood was twice as frequent as the blood of birds.

Mechanical Transmission of Sleeping Sickness by the Tsetse Fly.

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(Received June 22,—Read June 30, 1910.)

Up to the beginning of 1909 it was believed that the spread of trypanosome diseases, such as Sleeping Sickness and Nagana, was effected by the mechanical transmission of the parasite by the tsetse fly. The proboscis was supposed to be contaminated by being dipped in the infected blood, and some of the trypanosomes were pictured as remaining for some time within the tube and capable of being injected into a fresh animal at the next feed of the fly. Successful experiments were described which seemed to prove that the tsetse fly was capable of remaining infective for 48 hours, but not longer; and it was thought that any given Sleeping Sickness area would be free from danger a few days after the infected population had been removed from it.

Dr. Kleine, however, at the end of 1908, showed that the tsetse fly remains infective for a much longer period, and that a period of non-infectivity of 20 days or more elapses before this power of passing on the parasite is gained. In other words, that the *Trypanosoma gambiense* undergoes some process of development in the fly before it is able to infect a fresh animal. It was evident, then, that the mechanical theory had to be modified.

It was now held that, in addition to the mechanical method, a mode involving a developmental phase within the fly must be reckoned with. But it was still considered likely that the mechanical was by far the more common mode of infection, and that for every case due to a developmentally-infected fly, a hundred would be due to recent contamination. At the same time, it was quite evident, from an examination of the old feeding experiments after 8, 12, 24, and 48 hours, that the successful results recorded might have to be credited to late-infectivity rather than to mechanical transmission. The experiments lasted so long that there was enough time for the unsuspected late development of the parasite in the fly to have caused the infection.

The following experiments were therefore made to put this matter to the proof. The flies, after having had their infective feed, were, as a rule, not allowed to feed on a healthy animal for more than 12 days, in this way preventing the chance of the infection being transmitted by flies in which the development of *Trypanosoma gambiense* had taken place.

A. *Mechanical Transmission of* Trypanosoma gambiense. *Interrupted Feeding.*

In interrupted feeding, the cage containing the flies is first placed on the infected animal for some little time, then suddenly transferred to the healthy animal, and so backwards and forwards for 10 to 15 minutes. This is meant to imitate the conditions in Nature, when sick and healthy natives are sitting together on the Lake-shore, and the tsetse flies are continually flitting from one to the other.

Experiment 1550. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense.* From infected to healthy monkey.

Date.	Day.	Procedure.	Remarks.
1909.			
Aug. 30—31	1	Cage changed from infected to non-infected monkey every 15 seconds. This continued for 10 minutes each day.	65 flies used.
Sept. 1	2		
„ 2	3		
„ 3	4		
„ 4	5		Sept. 6, healthy monkey showed *Trypanosoma gambiense.*
„ 5	6		
„ 6	7		

Result.—Positive. The infection probably took place on the first day (August 30), as it usually takes seven days before the trypanosomes appear in the blood.

Experiment 1316. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense.* Infected ox to healthy monkey.

The two oxen used for this experiment were known to be infective, as their blood, when injected into a clean monkey, gave rise to a *gambiense* infection.

Date.	Day.	Flies fed on infected oxen and healthy monkey.	Alternate feeds.	Remarks.
1909.				
July 15—16	1	Flies fed daily	20	5 flies used.
„ 17—31	2—16	„ „	20	July 16, 4 flies added.
Aug. 1	17	„ „	20	Aug. 1, 20 flies added.
„ 2—Sept. 7	18—54	„ „	20	Sept. 7, 10 flies left; dissected; all negative. Monkey remained healthy.

Result.—Negative. The trypanosomes are usually very few and far between in the blood of cattle, which probably accounts for the negative result.

Experiment 1543. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*. Infected monkey to healthy goat.

Date.	Day.	Feedings from infected monkey to healthy goat.	Remarks.
1909.			
Aug. 28	—	20 alternate feeds.	50 flies used.
„ 29—30	1	25 „ „	
Sept. 9...............	2—12	Continued daily for 12 days.	

Result.—Positive. The goat, Experiment 1495, was examined daily for trypanosomes until October 16, but none was seen. On October 1, 21 days after the last feed of infected *Glossinæ palpalis*, 4 c.c. of the blood of this goat were injected into monkey, Experiment 1777. This monkey showed trypanosomes on October 16, proving that the goat, Experiment 1495, had become infected from the monkey by interrupted feeding, though trypanosomes failed to appear in its blood.

Experiment 1565. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*. Infected ox to monkey.

Date.	Day.	Interrupted feeding on infected ox and healthy monkey.	Remarks.
1909. Aug. 29—Sept. 9	1—11	25 passages to each animal, the whole lasting about half an hour. This continued daily for 11 days.	30 flies used. The monkey, examined for 30 days, failed to show trypanosomes.

Result.—Negative. Blood of ox injected into monkey on August 28. This monkey became infected with *Trypanosoma gambiense* six days later. The negative result may be due to the small numbers of trypanosomes found at any time in oxen.

Experiment 1705. Interrupted Feeding.

Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense*. Infected monkey to fowl.

Date.	Day.	Interrupted feeding on infected monkey and healthy fowl.	Remarks.
1909. Sept. 28—Oct. 4	1—6	30 passages from monkey to fowl, and continued daily for 6 days.	60 flies used.

Result.—Negative. October 7, 1 c.c. of blood of fowl injected into healthy monkey. November 2, 2 c.c. of blood of fowl injected into healthy monkey. Both monkeys remained healthy.

Table I.—Summary of Results of Interrupted Feeding Experiments.
Laboratory-bred *Glossina palpalis* and *Trypanosoma gambiense.*

Experiment.	Infected animal.	Healthy animal.	No. of days flies fed.	Result.		Remarks.
				Positive.	Negative.	
1550	Monkey ...	Monkey ...	7	+		65 flies used.
1316	Ox	„ ...	54		—	5 to 29 flies used.
1543	Monkey ...	Goat	12	+		50 flies used.
1565	Ox	Monkey ...	11		—	30 flies used.
1705	Monkey ...	Fowl	6		—	60 flies used.

B. *Mechanical Transmission of* Trypanosoma gambiense *by Laboratory-Bred* Glossina palpalis, *with an Interval between the Feeding of the Flies on the Infected and Healthy Animals.*

The following table gives a summary of the results :—

Table II.

Experiment.	No. of days flies fed.	No. of flies fed.	Infected animal.	Healthy animal.	Interval between feedings.	Result.	
						Positive.	Negative.
1712	12	50	Monkey...	Monkey...	½ hour		—
1233	12	14	„ ...	„ ...	1 „		—
1664	12	50	„ ...	„ ...	1 „		—
1665	12	40	„ ...	„ ...	2 hours		—
1524	11	35	„ ...	„ ...	6 „		—
1080	12	11	„ ...	„ ...	8 „		—
1801	13	70	„ ...	„ ...	24 „		—
1802	13	75	„ ...	„ ...	24 „		—
1803	13	120	„ ...	„ ...	24 „		—
1274	15	7	„ ...	„ ...	48 „		—

Conclusions.

1. The mechanical transmission of Sleeping Sickness by means of *Glossina palpalis* can take place if the transference of the flies from the infected to the healthy animal is instantaneous—that is, by interrupted feeding.

2. This mechanical transmission does not take place if an interval of time comes between the feedings.

3. Mechanical transmission plays a much smaller part, if any, in the spread of Sleeping Sickness than has been supposed.

The Chromaphil Tissues and the Adrenal Medulla.

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(Communicated by Prof. E. A. Schäfer, F.R.S. Received April 6,—Read April 28, 1910.)

(From the Physiological Laboratory, University of Manitoba, Winnipeg, Canada.)

[Plate 13.]

I. *Introductory and Historical.*

The recognition of the extra-adrenal chromaphil[*] cells in mammals and the characteristic reaction with chromium salts, by which they are now universally designated and homologised with the medulla of the adrenals, is due to Stilling.[†] This author found in the abdominal sympathetic small bodies composed of cells having the same chromaphil reaction as those forming the medulla of the adrenal. He states that some are nearly a centimetre in length, while others are only just visible to the naked eye. They are round, oval, or elongated in form, and their thickness is never more than a few millimetres. They have a *tunica propria*, small vessels and capillaries. Between the capillaries are cells which resemble in all respects those of the adrenal medulla. The resemblance between the chromaphil corpuscles of the sympathetic and the medulla of the adrenal is rendered all the greater by the occurrence in the latter of occasional nerve cells. Stilling found these corpuscles in the rabbit, the cat, and the dog, and especially in young animals. He gives details of a method for displaying them.

Stilling also discovered that the carotid body contains cells of the same character as those forming the adrenal medulla and the chromaphil corpuscles of the sympathetic.[‡] Later, Kohn[§] and Kose[||] confirmed and extended these observations, laying stress on the point that these chromaphil cells are common and typical elements of the mammalian sympathetic system. Kohn's view is that what is ordinarily called the cortex of the adrenal is in reality the only part which ought to be called adrenal at all, while the medulla is simply the "paraganglion suprarenale," a group of what he calls "chromaffine Zellen," which has become included in the adrenal.

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[*] The spelling "chromaphil" instead of chromophil was suggested to me by Prof. Schäfer.

[†] 'Revue de Médecine,' November, 1890, p. 808 ff.

[‡] 'Recueil inaugural de l'Université de Lausanne,' 1892.

[§] 'Prager med. Woch.,' 1898, Bd. 23, s. 194–195; 'Arch. f. mikr. Anat.,' 1898, Bd. 53, s. 281–312; 'Anat. Anz.,' 1899, Bd. 15; 'Ergebnisse d. Anat. u. Entwick.,' 1899, Bd. 9; 'Arch. mikr. Anat.,' 1900, Bd. 56; 'Prag. med. Woch.,' 1902, Jg. 27; 'Arch. f. mikr. Anat.,' 1903, Bd. 62; 'Allg. Wien. med. Zeitschr.,' 1903, Jg. 48, nr. 46 u. 47; 'Ergebnisse Anat. u. Entwickl.,' 1902, Bd. 12; 'Prag. med. Woch.,' 1903, nr. 42.

[||] 'Sitz. Ber. d. naturw.-med. Ver. f. Böhmen, Lotos,' 1898, No. 6; 'Anat. Anz.,' 1902, Bd. 22; *ibid.*, 1904, Bd. 25.

Zuckerkandl* in 1901 found in the retroperitoneal space at the origin of the inferior mesenteric artery a pair of large chromaphil bodies which he calls "Nebenkörper des Sympathicus." These he found constantly in the embryo and in the new-born human subject. Biedl and Wiesel† have demonstrated that extracts prepared from them have precisely the same powerful effect upon the blood pressure as have extracts of the medulla of the adrenal.

The extra-capsular chromaphil material is still very imperfectly known to many writers on the physiology and pathology of the adrenals. Thus Rolleston‡ in this relation refers to Zuckerkandl's bodies, the carotid body, the coccygeal body, and some cells in the pituitary. Now the coccygeal body does not contain chromaphil cells,§ nor does the pituitary, so far as I am aware. Zuckerkandl's description applies only to the human subject, and indicates, as we have seen, a *pair* of chromaphil bodies in the retroperitoneal region of the abdomen. But in the dog and other animals there is to be found a very striking mass (or more correctly a "strip") of chromaphil tissue (the "paraganglion aorticum abdominale" of Kohn). This is *unpaired*, and may be called the "abdominal chromaphil body." The existence of this important body is probably unknown to many physiologists and anatomists. Kohn‖ has given an excellent account of this body and the other chromaphil tissues in the human subject, and also in the cat and the rabbit, but his paper does not deal with these structures in the dog. In regard to the general anatomy of the chromaphil bodies in the dog the present communication cannot lay claim to any originality, for Kohn has been kind enough not only to explain to me how to find the bodies, but also to send me illustrative preparations.

The present paper will, I hope, be useful to experimenters and others, inasmuch as it is the only account in the English language of the chromaphil structures, and as it records the results of the examination of a large number of animals and contains a fuller description of the microscopic structure of the chromaphil bodies than has hitherto appeared.

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* 'Verhandl. d. Anatom. Ges.,' Bonn, 1901, 15 Vers.; 'Anat. Anz.,' Ergänzungsh. z. 1901, Bd. 19, pp. 95—107.

† 'Arch. f. d. ges. Physiol.,' 1902, Bd. 91.

‡ 'Lancet,' 1907, vol. 2, p. 875.

§ Stoerk, 'Arch. f. mikr. Anat.,' 1903, Bd. 69.

‖ 'Arch. f. mikr. Anat.,' 1903, Bd. 62.

II. *Methods Employed.*

For the study of the naked-eye anatomy of the mammalian chromaphil tissues the following method (of Kohn and Stilling) has been adopted:—The liver and alimentary tract are removed from the abdomen and a piece of absorbent cotton soaked in a solution of potassium bichromate (3·5 per cent.) is placed over the retroperitoneal tissues and left *in situ* for 6 to 12 hours. At the end of this (or in some cases a longer or a shorter time) the adrenals, aorta, vena cava, and subjacent and superjacent tissues are cut out as far back as the bifurcation of the aorta and washed in running water for several hours. The chromaphil bodies are then plainly seen, and still more plainly if the whole preparation be placed in glycerine. They appear as dark brown streaks, patches, or dots of varying size and shape (see text-figs. 1 to 11).

Another method which gives good results is to first cut out the abdominal vessels and the adrenals with the accompanying tissues and lay the preparation in a solution of potassium bichromate (3·5 per cent.). In rabbits and cats it is recommended that the animals chosen for examination be not too fat.

It is sometimes desirable to make a little further dissection after the action of the bichromate. To see the smaller bodies, a simple lens or a dissecting microscope may be employed. It is best that fresh material should be employed; but fairly good results may sometimes be obtained, even if the animal has been dead for some hours before dissection.

For the microscopical examination of the chromaphil cells and bodies in the first instance, Kohn's fluid (potassium bichromate, 3·5 per cent., 90 c.c.; formol, 40 per cent., 10 c.c.) has been employed, or the bodies revealed macroscopically by the application of bichromate have been washed, passed through alcohol, and treated by any of the usual methods for microscopical preparation.

But bichromate solutions, even with the addition of a certain proportion of formol, do not give the best results for detailed histological examination, howsoever convenient they may be for identification of chromaphil cells. For this reason, after the bodies have once been carefully located by means of the bichromate, from subsequent specimens they have been fixed in corrosive sublimate, Flemming's fluid, and other approved agents. The sections were stained in various ways. For the small groups of chromaphil cells in the sympathetic ganglia, it has been necessary to employ bichromate solution throughout, because otherwise it is almost impossible to recognise them in sections.

The following species have been examined:—Dog, cat, rabbit, guinea-pig, white rat, mouse, monkey (macacus), pig, gopher (*Spermophilus Franklini*), sciurus (species not identified).

III. *The Chromaphil Cells in the Sympathetic Ganglia.*

According to Kohn and Kose, groups of chromaphil cells are regularly found in the ganglia of the sympathetic chain, and in numerous ganglia and nerves of the peripheral sympathetic plexuses.

V. Ebner,* in reference to these cells, speaks doubtfully, "nach eigenen, allerdings nur flüchtigen Beobachtungen kann ich vorläufig an das regelmässige Vorkommen von 'chromophilen' oder 'chromaffinen' Zellen in den Ganglien des Sympathicus bei Sängern nicht glauben und halte vor allein nicht für erwiesen, dass die in Chromsalzen sich gelb färbenden Zellen des Sympathicus mit den Markzellen der Nebenniere identisch sind, da diese Farbenreaktion für sich allein nicht beweisend ist." He points out with perfect justice that in order to prove that these groups of cells are identical with those of the adrenal medulla, something further than the chrome reaction is necessary, and remarks that too much stress should not be laid upon the common origin of the adrenal medulla and these cells from the sympathetic.

I must confess to having felt very similar doubts at an early stage of the investigation. But although these chromaphil cells in the sympathetic ganglia are not, perhaps, so common as one might be led to conclude from a perusal of Kohn's papers, yet if a large number of sections be cut, one can scarcely fail to find them, at any rate in the larger ganglia.

Plate 13, fig. 3, shows a section through a group of chromaphil cells in the inferior cervical ganglion of a dog. The tissue was fixed in Kohn's fluid, and the sections were stained with hæmalum. The group of cells measures 0·12 mm. by 0·1 mm., and stands out prominently from the nervous tissue by reason of the brown stain of the cytoplasm, and in many cases of the nucleus also.

The mass of cells is not divided up into distinct columns and smaller masses as is the case with the abdominal chromaphil body (*cf.* fig. 4), and the adrenal medulla (*cf.* figs. 2 and 5), but there are traces of such division indicated by wavy lines which have not taken on the chrome reaction (see fig. 3).

The cell-outlines are for the most part distinctly seen, and are indicated in many cases by a light (unstained) ring, which is again surrounded by a dark, slightly irregular border (Plate 13, fig. 3, *chrom. c*). The cells are about 15 μ in diameter, and the protoplasm is very finely granular. The brown tint varies in intensity in different cells and in different parts of the same cell.

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* Kölliker's 'Handb. d. Gewebelehre,' 1902, 6te Aufl.

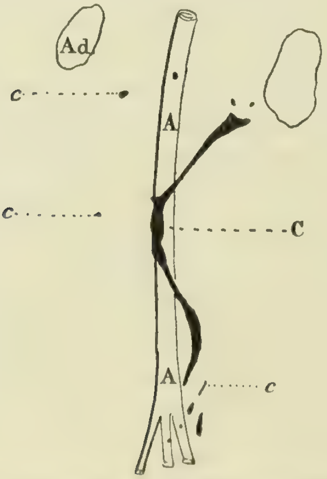
The nuclei are large in proportion to the dimensions of the cells, and are on an average 7 μ in diameter (see fig. 3, *n*). The majority are stained brown by the bichromate, and the outlines are distinct and dark brown, while the interior is usually clearer. There is sometimes a distinct nucleolus, and the outer portion of the nucleus is studded with small dark brown granules (fig. 3, *n*).

Nuclei belonging to nerve-fibres are also to be seen within the area of the group of chromaphil cells (fig. 3).

IV. *The Abdominal Chromaphil Body and other Accumulations of Chromaphil Cells.*

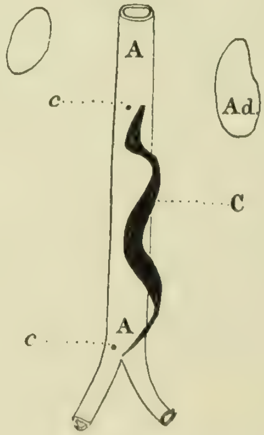
a. *Macroscopic.*

In the *dog*, the extra-adrenal chromaphil tissues are specially abundant, and the abdominal chromaphil body is easily and beautifully shown by the method above described. A few minutes after the mop of absorbent cotton soaked in bichromate has been placed over the aortic region, a long brown streak of rapidly deepening tint may be seen lying over the aorta, or slightly to one or the other side. In from half an hour to an hour its deep



TEXT-FIG. 1.—Abdominal Chromaphil Body of an Adult Dog.

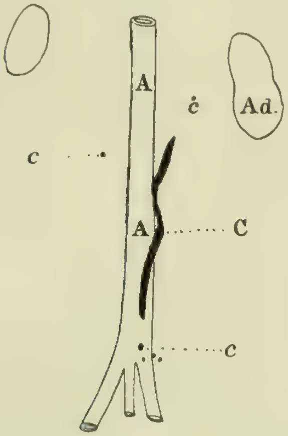
A = Aorta; Ad. = Adrenal; C = Abdominal chromaphil body; *c* = Smaller chromaphil bodies.



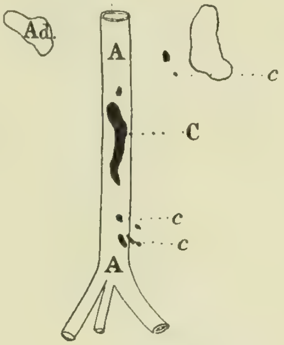
TEXT-FIG. 2.—Chromaphil Body of Adult Dog. Same lettering as text-fig. 1.

brown stain is fully developed, and the relations of the body can be determined.

It varies in length in the dogs I have dissected from 1 to 4·5 cm., and its width varies from a very fine line to about 5 mm.



Text-fig. 3.—Chromaphil Bodies of an Adult Dog. Lettering as in previous figures.



Text-fig. 4.—Chromaphil Bodies of a Young Dog. Lettering as in previous figures.

Some illustrations of the variations in size and shape which may be met with are furnished by the text-figs. 1 to 4. The body is flattened dorso-ventrally, so that its thickness is not more than 0·2 to 0·3 mm. It varies considerably in width from place to place, is frequently branched, and shows broken-off portions. The principal body is unpaired.

Numerous irregularly placed small nodules of chromaphil tissue are seen in different regions in more or less close connection with the principal chromaphil body (see text-figures 1 to 4, *c*).

In the *cat*, the chromaphil bodies are not so large and dense as in the dog. They have been fully described by Kohn*, and, as will be seen from his drawings, as well as from those here given (text-figures 5 to 9), consist of long threads of tissue instead of ribbons of varying width as in the dog. These threads are stretched out along the sympathetic nerves, and the relation of the chromaphil tissue to the sympathetic is closer, or at any rate more obvious, than in either the dog or the rabbit. Small scattered chromaphil corpuscles are found in different regions, as in the dog (text-figures 5, 6, 7, 8, *c*).

In the *rabbit*, the threads of chromaphil tissue are thicker than in the cat.

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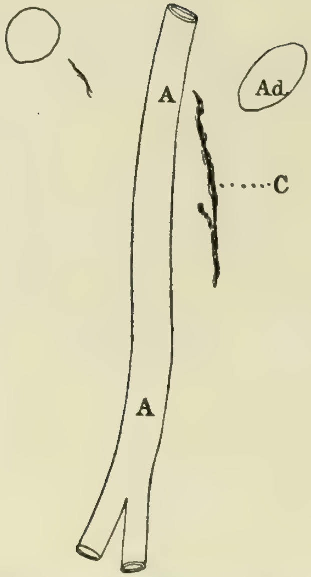
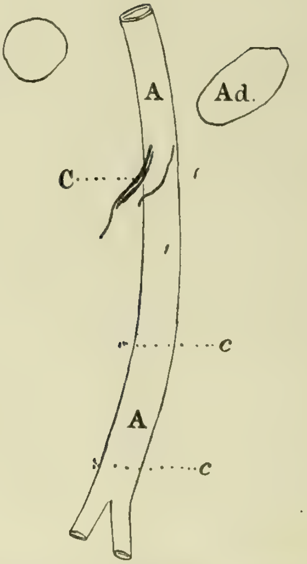
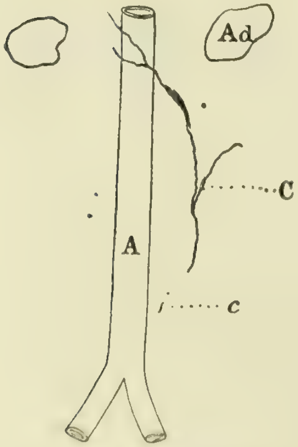
* 'Arch. f. mikr. Anat.,' 1903, Bd. 62, s. 317 ff.



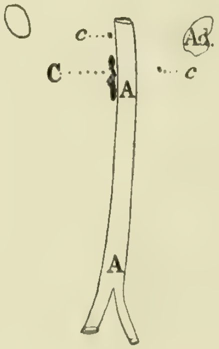
Text-fig. 5.—Abdominal Chromaphil Bodies of an Adult Cat. Lettering as in previous figures.



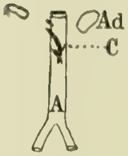
Text-fig. 6.—Abdominal Chromaphil Bodies of an Adult Cat. Lettering as in previous figures.



Text-fig. 7.—Chromaphil Bodies of Adult Cat.

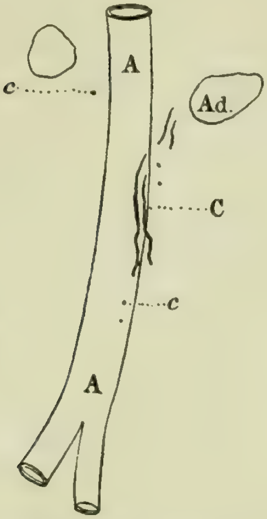


Text-fig. 8.—Chromaphil Bodies of Young Cat.

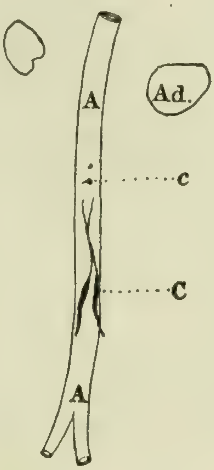


Text-fig. 9.—Chromaphil Bodies of New-born Kitten.

There is a tendency for the principal chromaphil body to be paired (text-fig. 10). Where this is not the case it is frequently bifurcated, anteriorly and posteriorly (text-fig. 11). The threads frequently run close to the adrenal, and are occasionally continuous with the medullary substance. This



TEXT-FIG. 10.—Abdominal Chromaphil Bodies of a Rabbit. Lettering as in previous figures. The principal body is paired, as also is a continuation towards the adrenal on one side.



TEXT-FIG. 11.—Abdominal Chromaphil Bodies of a Rabbit. Lettering as in previous figures. The principal body is bifurcated anteriorly and posteriorly.

is shown in one of Kohn's drawings, and corresponds with what I found some years ago in the young rabbit. I described the appearance as follows:—"In young animals one sometimes finds that the medulla has not yet become completely surrounded by cortex, but comes to the surface at some point. In the case of the suprarenal capsule of a young rabbit, I noted a very interesting appearance. Near that part of the circumference where the medulla reached the surface was a sympathetic ganglion outside the capsule of the organ. Near to it, also outside the capsule, was a mass of cells resembling those of the suprarenal medulla, only not so uniformly stained by the bichromate."* This was clearly a group of chromaphil cells, and if serial sections had been cut, doubtless a direct continuity between it and the medulla would have been seen.

In the other animals examined, viz., monkey, pig, guinea-pig, rat, gopher and squirrel, no chromaphil bodies have been recognised with certainty. This is especially interesting in the case of the rat. Schäfer† recently exhibited a white rat which was operated upon by Harley some time between 1856 and 1858. This rat had the spleen and adrenals extirpated when it was only a month old and quite small. It increased in size after the operation quite as fast as its fellows which had not been touched. The animal was killed when five months old, and no discoloration of the skin or hair could be detected. Commenting upon this Prof. Schäfer says "the rat happens to be the one common animal which is able to withstand complete removal of both suprarenal capsules. The reason for this was not at the time apparent, although it is now known, for the rat is exceptional in possessing in various parts of the back of the abdomen and pelvis numerous small glandular structures which are composed of cells having the characteristic features and functions of the cells of the suprarenal medulla."

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* 'Internat. Monatsschr. f. Anat. u. Physiol.,' 1898, Bd. 15, Heft 10 u. 11.

† Oliver-Sharpey Lectures, 'Brit. Med. Journ.,' May 30, 1908.

I have carefully examined several white rats, but have failed to find any traces of extra-adrenal chromaphil tissue. There is probably, however, some difference in this respect between the different varieties of rats, for Dr. Kohn informs me that he has found an abdominal chromaphil (paraganglion aorticum abdominale) in a rat which he dissected, and he has sent me an illustrative sketch. But he is of opinion that the rat does not possess a greater abundance of chromaphil tissue than other animals.

From the large size of the chromaphil body in the dog one would expect to find this animal more resistant to adrenal extirpation than other animals, if the chromaphil tissue be looked upon as essential to life. But the experimental evidence bearing upon the relative importance to life of cortex and medulla is very contradictory.*

b. *Microscopic.*

My investigations upon the minute structure of the abdominal chromaphil bodies have been confined to the dog. In this animal the body is large enough to be found without treatment with bichromate, and so can be dissected out and fixed in any fluid one chooses.

In preparations made with bichromate solutions (*e.g.*, Kohn's fluid, *vide supra*) the chromaphil cells are stained of a tint which varies from a light yellow to a deep brown, according to the duration of the treatment with the solution, and apparently also according to physiological conditions in the cells.

Plate 13, fig. 4, shows a transverse section through the abdominal chromaphil body (dog) fixed in Kohn's fluid. The section is seen to be divided up into a number of irregular areas which represent the cross-sections of columns of cells which run longitudinally. The width of these columns varies considerably (see Plate 13, figs. 1 and 4). They are divided from each other by strands of connective tissue with a large amount of elastic tissue and blood-vessels and nerves. In the spaces between the columns is also accumulated a quantity of homogeneously stained material which appears to resemble the colloid of the thyroid. This seems to be in some instances contained in lymph spaces.

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* For a discussion of this subject see Vincent, 'Ergebnisse der Physiol.,' 1910.

The cells are irregular in shape (Plate 13, fig. 4), but tend, like those of the chromaphil groups in the sympathetic ganglia, to be circular in outline (*cf.* Plate 13, fig. 3 and fig. 4, *chrom. c*). They have an average diameter of about 12 μ. Thus they are slightly smaller than those in the ganglia.

The protoplasm is very finely granular or almost homogeneous, and the intensity of the brown coloration varies considerably in different cells (Plate 13, fig. 4), so that their outlines are often indicated by this means. Some cells scarcely show the brown coloration, and the region of the protoplasm is indicated as a light ring round the nucleus.

A very striking feature of the preparation is the appearance in all parts of the section of a number of clear spaces with very definite outlines (Plate 13, fig. 4, *v*). These appear in many instances to contain a substance resembling the colloid above referred to, and occur both between the cells and within their substance.

The nuclei are 5 or 6 μ in diameter, and resemble those of the groups of chromaphil cells within the ganglia (Plate 13, figs. 3, *n*, and 4). In many of my slides a very interesting arrangement is seen. The nuclei of the cells are frequently disposed in a regular row around the circumference of the cell-columns.

When the material is fixed in corrosive sublimate and subsequently stained with hæmalum or carmalum many of the features above described are seen with greater distinctness. Thus the cell outlines are more regularly and more distinctly seen, and the shape and relations of the cell-columns are more easily studied. Specimens of the abdominal chromaphil body of the dog have been removed immediately after the death of the animal, and after being pinned out on cork to prevent shrinking or curling have been fixed in acid mercuric chloride solution. Sections have been subsequently cut in three directions, viz., transversely, longitudinally and horizontally, and longitudinally and vertically (Plate 13, fig. 1).

The organ is surrounded by a connective tissue capsule containing vessels and nerves (fig. 1, *c*). It is clear that the cell-columns run longitudinally, and are somewhat wider in the horizontal than in the vertical direction. There is considerably more variation in the width of the columns in a horizontal direction than in the vertical. In the former direction the columns have an average width of 52 μ, in the latter about 22 μ. The average size of cells and nuclei is given above in connection with the bichromate preparations.

The vacuoles so prominent in bichromate specimens are not to be seen in the preparations fixed in corrosive sublimate or Flemming's fluid (*cf.* fig. 1 with fig. 4).

As already pointed out by Kohn,* the abdominal chromaphil bodies occur in two principal forms, the globular and the elongated. These last are often in the form of threads. The first form is typically found in the human subject; the last in other mammals, such as cats and rabbits. In these animals the threads or columns run separately for the most part, while, as we have seen, in the dog they are united into a compact ribbon-like organ.

In the fœtal kitten, the chromaphil body is short, almost oval in shape, and the columns of chromaphil cells form an irregular network. Kohn gives an excellent drawing of the microscopical structure of this body, so that a description in this place is unnecessary.

V. *The Adrenal Medulla.*

There is no need to give a description of the general structure of the medulla of the adrenal. The structure has been described by numerous observers, and is generally known. It is, however, desirable to institute some comparisons between the histological appearances of the adrenal chromaphil tissue and this substance as it occurs in other places, as, for example, in the sympathetic ganglia and in the abdominal chromaphil bodies.

The present observations are confined to the adrenal of the dog, just as the descriptions of the abdominal chromaphil body are limited to this animal.

A comparison of Plate 13, fig. 1, with fig. 2 or of figs. 3 and 4 with fig. 5, will show that the general resemblance between extra-adrenal chromaphil tissue and adrenal medulla is very great. Both consist of columns of cells staining yellow or brown with bichromate of potash. The cell-columns are, however, for the most part much thicker in the adrenal than in the abdominal chromaphil bodies. The blood-spaces are wider, and the whole aspect gives the impression that the adrenal medulla is more highly organised (see Plate 13, fig. 2, *bld. v*, *col. c*).

Many of the cells of the adrenal medulla are spherical, as in the abdominal chromaphil body, and their dimensions are the same, viz., about 12 μ in diameter. The nuclei, also, are of the same order of magnitude in both structures, viz., 5 or 6 μ. But in many regions, especially where the cell-columns are separated by large venous sinuses, the cells are arranged in a definitely epithelial fashion round the blood-vessels (Plate 13, fig. 2, and fig. 5, *col. c*). In this case the cells are columnar in shape, and may reach a length of 26 μ, and the nuclei are placed at the end of the cell remote from the blood-vessel.

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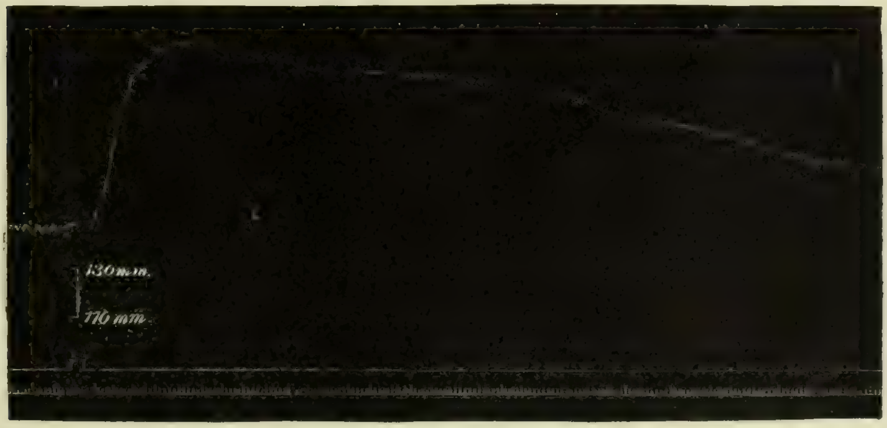
* 'Arch. f. mikr. Anat.,' 1903, Bd. 62, s. 327.

The protoplasm of the adrenal medulla is more distinctly granular than that of the abdominal chromaphil body, and is, moreover, more delicate in consistence, and therefore shows more shrinkage in fixation and tearing during the process of cutting sections. When the adrenal is fixed in bichromate solutions, the section shows vacuoles similar to those described above in the chromaphil body. These are absent in sublimate and Flemming preparations. Thus it seems justifiable to regard the medulla of the adrenal body as composed of chromaphil cells of the same general character as those forming the chromaphil bodies. But the former have undergone specialisation, and the structure of the substance has become elaborated into an organ with more definitely glandular form.

VI. *The Question as to the Secretory Activity of the Chromaphil Tissues.*

It is clear from all that has gone before that the extra-adrenal chromaphil tissues contain a substance which gives the same macro- and micro-chemical reactions as adrenin. It has been shown by Biedl and Wiesel* that the "parasomata" (Nebenkörper) discovered by Zuckerkandl in the human subject contain adrenin or some substance which has an identical effect upon the blood-pressure. I have recently been able to prove that the abdominal chromaphil bodies of the dog contain the same or a similar substance. Text-fig. 12 shows the effect of injecting into the saphenous vein of a dog an



Text-fig. 12.—Dog, 8 kilogrammes. November 10, 1909. $CHCl_3$, morphia, atropine. Carotid blood-pressure. Time in seconds. At the point signalled an extract from the chromaphil bodies of three dogs was injected into the saphenous vein.

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* 'Arch. f. d. ges. Physiol.,' 1902, Bd. 91.

extract made from the abdominal chromaphil bodies of three dogs. It will be seen that the effect is identical with that of adrenin. In the same experiment, a further injection of an extract made from the abdominal chromaphil bodies of two dogs produced a comparable rise of blood-pressure.

Mulon* has raised the blood-pressure of an animal by injection into the circulation of an extract made from the carotid body of the horse.

It seems clear, therefore, that all chromaphil tissues, whether contained in the adrenal or not, yield adrenin, or a substance having similar chemical and pharmacodynamical properties. According to the views of the majority of recent observers, the presence of this substance may be considered, in conjunction with other observations, as evidence of an internal secretion on the part of all these tissues. Kohn,† from morphological considerations, is opposed to this view. He is certainly correct in insisting that because adrenin has certain pharmacodynamic properties we have no right to assume, without further definite evidence, that it is one of the functions of the tissue to pour out this substance into the blood-stream in order to produce beneficial effects upon certain tissues of the body. He considers that the cells forming the medulla of the adrenal and chromaphil tissue elsewhere are not "epithelial," and therefore cannot secrete. The present writer has replied to this argument on a previous occasion,‡ and a fuller discussion of the subject has recently appeared.§ It must be admitted that the experimental evidence on the point is contradictory, but there seems no reason why one cannot admit the hypothesis that all the chromaphil cells have an internal secretion, though this process is more completely elaborated in the large chromaphil bodies, and more especially in the adrenal medulla.

The presence of a large strip of chromaphil tissue in the abdomen of the dog and other animals, outside of the adrenals, is a very important consideration in all extirpation experiments, and the wide distribution of this tissue in the body renders its complete removal impossible.

EXPLANATION OF PLATE 13.

(Lettering common to all the figures.)

bld. c., blood corpuscles ; *bld. v.*, blood vessels ; *c.*, capsule ; *chrom. c.*, chromaphil cells ; *col. c.*, columnar cells of adrenal medulla ; *conn. t.*, connective tissue ; *end.*, endothelium of blood vessels ; *n.*, nuclei ; *n.c.*, nerve cells ; *n.f.*, nerve fibres ; *v.*, vacuoles.

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* 'Arch. gén. de Méd.,' 1903, Année 81, T. 2, No. 52.

† *Loc. cit.*

‡ 'Anat. Anz.,' 1900, Bd. 18.

§ Vincent, 'Ergebnisse der Physiol.,' 1910.

Fig. 1.—Transverse section through the abdominal chromaphil body of the dog. Fixed in corrosive sublimate and stained with hæmatoxylin. Section $10\,\mu$ in thickness. Leitz, obj. 6. Drawing ocular.

Fig. 2.—Section through the medulla of the adrenal of a dog, prepared as in case of previous figure. Same magnification.

Fig. 3.—Section through a group of chromaphil cells in the inferior cervical ganglion of the dog. Fixed in Kohn's fluid (bichromate-formol, see text). Leitz, $\frac{1}{12}''$ oil immersion. Drawing ocular.

Fig. 4.—Transverse section through the abdominal chromaphil body of the dog. Fixed in Kohn's fluid (*v. supra*), stained with hæmatoxylin. Leitz, $\frac{1}{12}''$ oil immersion. Drawing ocular.

Fig. 5.—Section through the medulla of the adrenal of a dog. Kohn's fluid. Hæmatoxylin. Leitz, obj. 6. Drawing ocular.

On the Occurrence of a Mesocœlic Recess in the Human Brain, and its Relation to the Sub-Commissural Organ of Lower Vertebrates; with special reference to the Distribution of Reissner's Fibre in the Vertebrate Series and its possible Function.

By Arthur Dendy, D.Sc., F.R.S., Professor of Zoology in King's College (University of London), and G. E. Nicholls, B.Sc., Assistant-Lecturer and Demonstrator in Zoology in King's College.

(Received May 24,—Read June 2, 1910.)

[Plate 14.]

I. *Introductory.*

Eight years ago one of us (Dendy, 1902) published in the 'Proceedings' of the Royal Society a description of a peculiar structure lying beneath the posterior commissure in the brain of the Ammocœte, in the shape of a pair of longitudinal grooves lined by greatly elongated and apparently ciliated columnar cells, and evidently formed by specialisation of the ependymal epithelium which lines the cavity of the brain. Previous to this time there appear to have been, at the most, only a few scattered references to the occurrence of any such structure in the vertebrate brain. We now know, however, that it occurs throughout the whole vertebrate series, from the lampreys to the primates, it having recently been figured, in the case of *Macacus*, by Sir Victor Horsley (1908).

Sargent (1903) has given to this structure the name "Ependymal Groove," and has described it in a number of Ichthyopsidan types. He says, however (1903, 1904), that it is inconspicuous in mammals. We ourselves have recently observed it in a number of different forms. It is very well developed, for example, in Sphenodon, and we find it also strongly developed in the mouse and the cat. Sargent showed, further, that the "ependymal groove" is intimately connected with the anterior end of Reissner's fibre, and in fact regarded it merely as a kind of attachment plate for the latter. We also have been able to demonstrate quite clearly its connection with Reissner's fibre in a large number of types, *e.g. Geotria* (Dendy, 1907) and *Rana* (Nicholls, 1908). We shall discuss its possible function later on in the present communication; but, in the meantime, we may state that we do not consider that the term "ependymal groove" is sufficiently distinctive for so remarkable and constant a feature of the vertebrate brain, for it is, of course, not the only ependymal groove present. Inasmuch as it lies beneath the posterior commissure, we propose to speak of it in future as the "Sub-Commissural Organ." It appears primarily to be made up of two bands of columnar epithelium, usually more or less completely united together in the form of a groove; but, whereas it remains throughout life in a well developed condition in all the lower vertebrates, in man, as we shall endeavour to show in the present communication, it becomes reduced in the adult to a mere vestige sunk in the brain tissue at the back of the posterior commissure, but unmistakably recognisable as the homologue of the sub-commissural organ of lower types.

II. *The Sub-Commissural Organ in the Mouse, Cat, and Chimpanzee.*

As the sub-commissural organ is as yet very little known, we propose, for purposes of comparison, to give a short description of it as it occurs in the mouse, the cat, and the chimpanzee, which form a series leading up to the vestigial condition in the human brain. We believe the organ in question has never yet been described in any of these types, nor, indeed, has a complete description of it been given for any mammal.

Mouse.—In a sagittal section of the brain of the common house-mouse, such as is represented in text-fig. 1, we see the infra-pineal recess (*i.p.r.*) projecting upwards and backwards between the posterior (*p.c.*) and superior (*s.c.*) commissures, and leading to the elongated pineal gland (*p.g.*). In front of the superior commissure the roof of the third ventricle is produced upwards and backwards to form the supra-pineal recess (*s.p.r.*), a narrow diverticulum which lies parallel to the infra-pineal recess and pineal gland, and which is doubtless homologous with the dorsal sac of lower types. The wall of the supra-pineal recess is greatly folded to form a choroid plexus (*c.p.*), and above and below it lie large veins (*v*). The lower surface of the posterior commissure is covered, in the middle line, by the peculiar high columnar

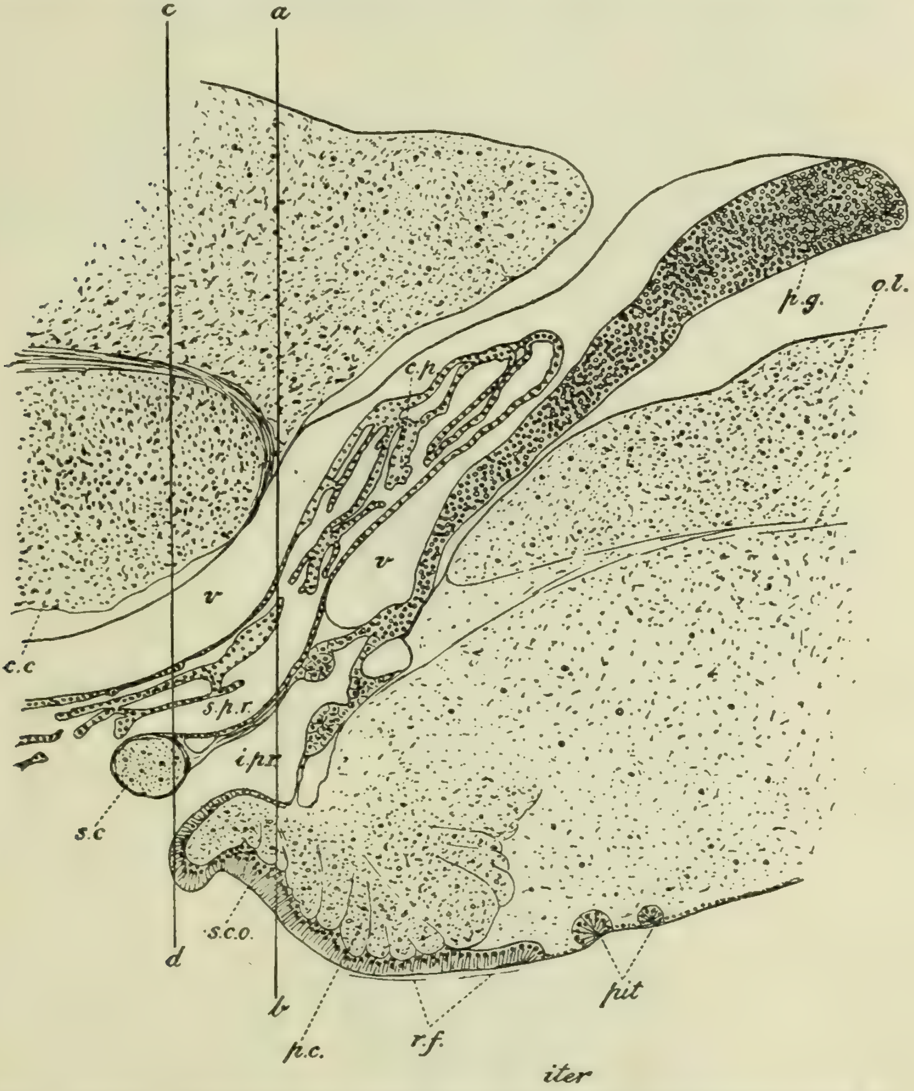

FIG. 1.—Mouse. Sagittal Section through the Region of the Posterior Commissure, &c. × 60. (The lines *ab*, *cd*, indicate approximately the planes of the transverse sections represented in figs. 2 and 3.)

c. c., corpus callosum ; *c. p.*, choroid plexus ; *i.p. r.*, infra-pineal recess ; *o. l.*, optic lobe ; *p. c.*, posterior commissure ; *p. g.*, pineal gland ; *pit*, isolated invaginated patches of the epithelium of the sub-commissural organ ; *r. f.*, Reissner's fibre ; *s. c.*, superior commissure ; *s.c. o.*, sub-commissural organ ; *s.p. r.*, supra-pineal recess ; *v.*, veins.

epithelium of the sub-commissural organ (*s.c.o.*), which is continued round the anterior end of the posterior commissure for a short distance on to the floor of the infra-pineal recess, where it gradually loses its columnar character. Beneath the epithelium are seen portions of Reissner's fibre (*r.f.*), whose branches, no doubt, are connected with the epithelial cells as in lower types, and as in the cat to be described later on.

It will be noticed that the epithelium of the sub-commissural organ is thrown into a transverse fold near its anterior end, just before it turns round over the anterior end of the posterior commissure. Towards its hinder extremity it appears to become discontinuous, and two little separate islands

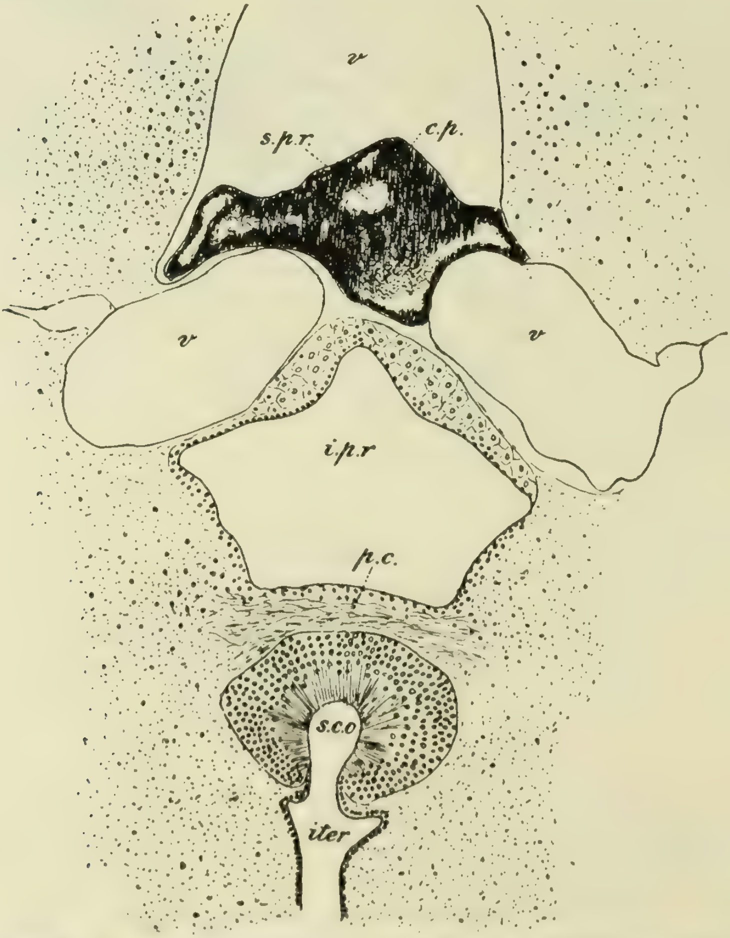

FIG. 2.—Mouse. Transverse Section through the Region of the Posterior Commissure, taken at about the level of the line *ab* in fig. 1. × 105.

c.p., choroid plexus; *i.p. r.*, infra-pineal recess; *p. c.*, posterior commissure; *s.c. o.*, sub-commissural organ; *s.p. r.*, supra-pineal recess; *v.*, veins.

are formed, which have become partially invaginated into the underlying tissues of the brain in the form of small pits (*pit*).

The transverse sections represented in text-figs. 2 and 3 will serve to further elucidate the relations above described. The lines *ab* and *cd* in text-fig. 1 indicate approximately the levels at which these two sections are taken (all the sections are, of course, drawn from actual preparations). In these sections we may confine our attention to the sub-commissural organ (*s.c.o.*). Text-fig. 2 shows this organ in the form of a very conspicuous horseshoe-shaped groove, lined by greatly elongated columnar cells and very sharply marked off from the ordinary ependyma of the *iter* below it, the latter

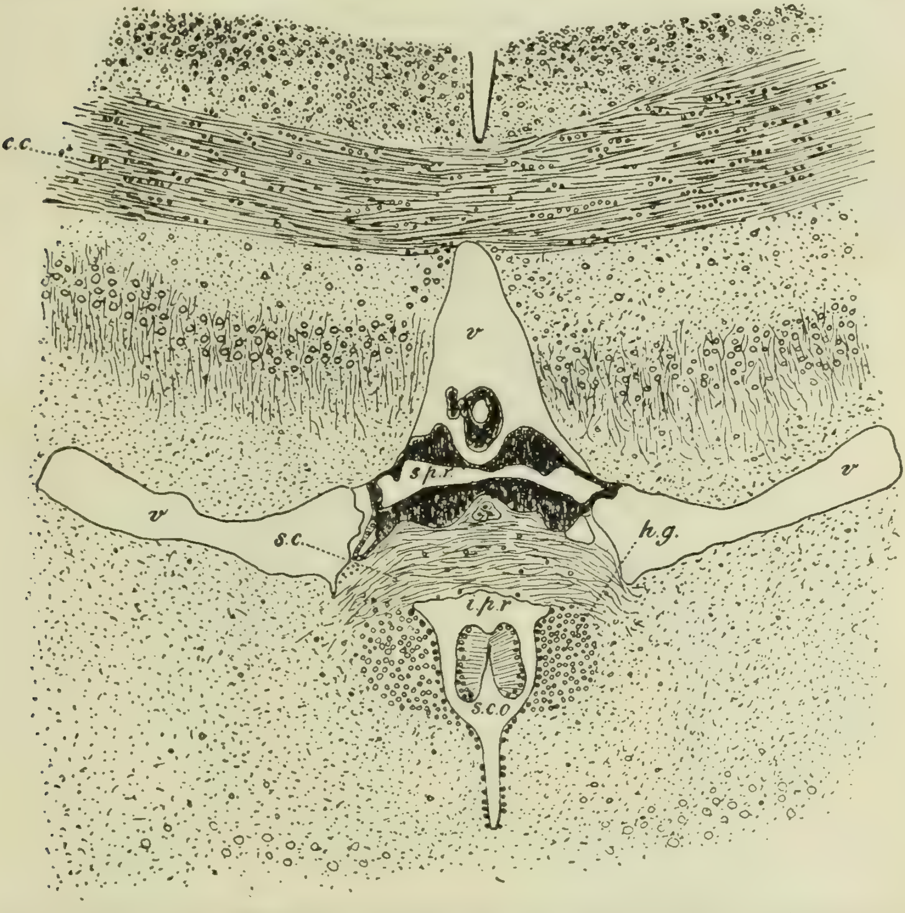

Fig. 3.—Mouse. Transverse Section through the region of the Superior (Habenular) Commissure at about the level of the line *cd* in fig. 1. × 60.

c. c., corpus callosum; *h. g.*, habenular ganglion; *i.p. r.*, infra-pineal recess; *s. c.*, superior commissure; *s.c. o.*, sub-commissural organ; *s.p. r.*, supra-pineal recess; *v.*, veins.

exhibiting a characteristic fold on either side where it joins the former. The cells of the sub-commissural organ are probably provided with cilia, but this is not evident in our preparations. The epithelium shows a strong tendency to split longitudinally at the bend of the horseshoe, which possibly indicates its paired character. A further indication of this paired character is seen in the section represented in text-fig. 3, where the epithelium is cut through quite separately and in two almost detached patches, as it projects from the anterior surface of the posterior commissure.

It is thus evident that in the mouse the sub-commissural organ is a very conspicuous structure, which probably has some important function connected with the associated Reissner's fibre.

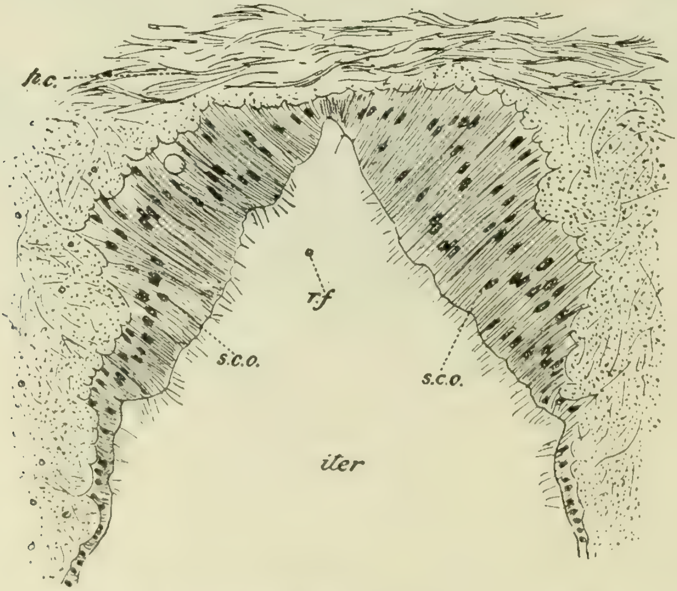

Fig. 4.—Cat. Transverse Section through the Sub-Commissural Organ in the Region of the Posterior Commissure. × 320.

p. c., posterior commissure ; *r. f.*, Reissner's fibre ; *s.c. o.*, sub-commissural organ.

The Cat.—In the cat the sub-commissural organ is not so well defined as it is in the mouse, but still very obvious, as will be seen from the transverse section represented in text-fig. 4. Here the double character of the organ is very well seen ; it is clearly made up of two bands of greatly elongated ependymal cells, right and left, and these cells appear to be distinctly ciliated. Reissner's fibre (*r.f.*), which can be traced for a long distance through this series of sections, is clearly seen lying in the groove between the two halves of the sub-commissural organ. Text-fig. 5 represents another section from the same series, but near the anterior end of the sub-commissural organ. The epithelium is seen to be losing its characteristic columnar appearance, especially in the middle line, but Reissner's fibre is still present, and, indeed, in this and neighbouring sections may be seen breaking up into branches, which actually run to the epithelium.

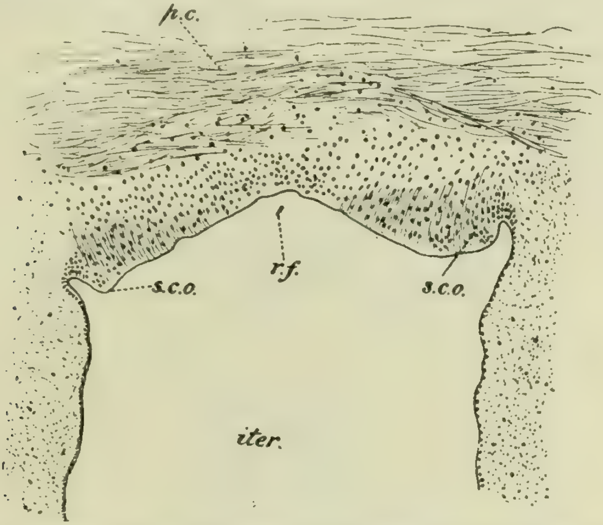

Fig. 5.—Cat. Transverse Section through the Region of the Posterior Commissure considerably anterior to the section shown in fig. 4. × 125.

p. c., posterior commissure ; *r. f.*, Reissner's fibre ; *s.c. o.*, sub-commissural organ.

The Chimpanzee.—Here, again, we have studied the sub-commissural organ by means of serial transverse sections. The entire brain had been hardened *in situ* by means of formalin and alcohol for ordinary anatomical purposes. It is naturally not in a good condition for the study of minute histological details, but shows very clearly the general form and relations of the parts under discussion.

The sub-commissural organ commences, as in the mouse, on the antero-dorsal aspect of the posterior commissure, where the latter forms the floor of the infra-pineal recess (compare text-fig. 1), where, however, it is very feebly developed. It can be followed backwards beneath the posterior commissure as a broad and very shallow groove (Plate 14, fig. 1, s.c.o.), the epithelium of which shows the nuclei arranged in several layers, but does not exhibit the typical differentiation until we come to about the hinder limit of the posterior commissure (text-fig. 6). Here the sub-commissural organ takes the form of two separate bands of the characteristic high columnar epithelium, which become invaginated into the roof of the *iter*, just behind the posterior commissure. This invagination (*m.r.*) turns forwards above the posterior commissure, where it is seen in the transverse section represented in Plate 14, fig. 1, the two epithelial bands having met together and completely surrounded the recess thus formed. This recess ends blindly in front at a distance of about 0·225 mm. from the point where it turns forwards above its opening into the *iter*. It has an internal diameter of about 0·25 mm. The enormously elongated, almost fibre-like cells by which it is surrounded measure about 0·057 mm. in length, and their nuclei are situated towards their inner ends. Though not in a good state histologically, it is evident that they agree closely in character with the corresponding cells in the cat and mouse.

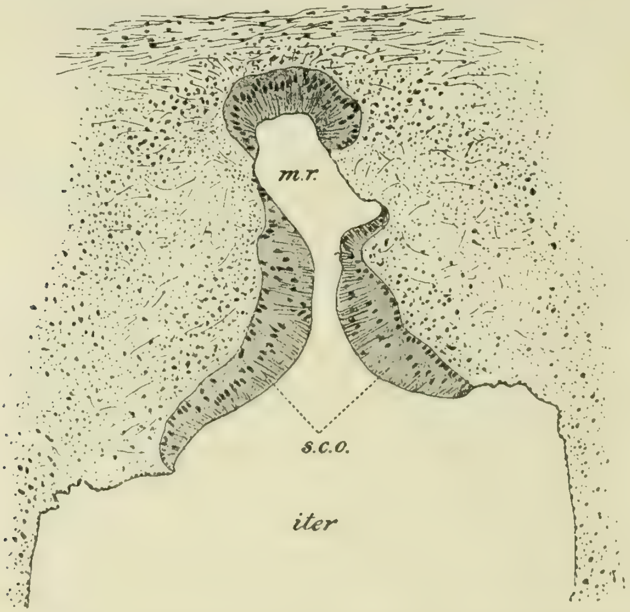

FIG. 6.—Chimpanzee. Transverse Section through the Sub-Commissural Organ at the Posterior End of the Posterior Commissure. × 60.
m. r., mesocœlic recess ; *s.c. o.*, sub-commissural organ.

This recess, which forms a very conspicuous feature in transverse sections of the chimpanzee's brain, corresponds in position with what Sargent (1904) has termed the *recessus mesocœlicus* in the sea lamprey (*Petromyzon marinus*), where also he regards it as part of the sub-commissural organ (Ependymal Groove). Stieda long ago (1870, fig. 15) figured a similar recess above the posterior commissure in the frog's brain, but without recognising its significance. Gaupp (1899, fig. 17) also figures this recess in the frog, and terms it the "diverticulum impar."

We have already noticed in the mouse a tendency on the part of the epithelium of the sub-commissural organ to become invaginated behind the posterior commissure, so that this tendency would appear to be very general throughout the vertebrate series. It is possible that in all cases the invagination may be apparent rather than real, and due to overgrowth of the sub-commissural organ by the surrounding tissues.

We have found no trace of Reissner's fibre in the chimpanzee, but it was hardly to be expected in material preserved merely for anatomical purposes.

III. *The Sub-Commissural Organ in Man.*

A mesocœlic recess, evidently homologous with that of the chimpanzee, but very much smaller, is readily recognisable in transverse sections of the adult human brain, where it appears to constitute the last vestige of the sub-commissural organ, while in the five months' fœtus this organ is still fairly well developed.

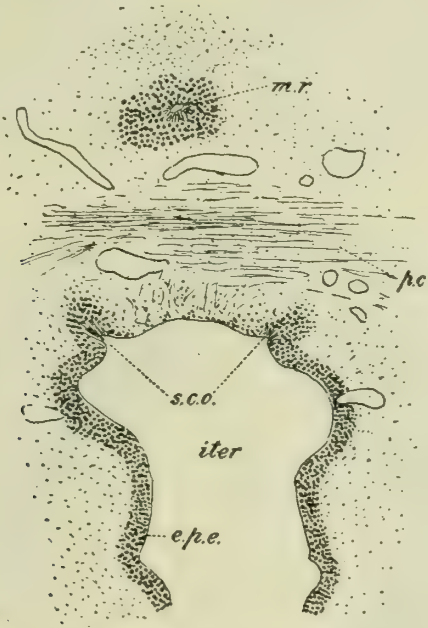

Fig. 7.—Human fœtus of about five months. Transverse section through the Region of the Posterior Commissure. × 60.

e.p. e., ependymal epithelium of *iter* ; *m. r.*, mesocœlic recess ; *p. c.*, posterior commissure ; *s.c. o.*, sub-commissural organ.

In the fœtus the sub-commissural organ is, indeed, rather better developed than in the chimpanzee. As in that animal, it begins anteriorly in the infra-pineal recess, where the characteristic high columnar epithelium, with deeply-seated nuclei, is well marked. As it is traced backwards around the curve of the posterior commissure its paired nature becomes evident. The two halves diverge, and come to lie widely separated in the roof of the *iter*, forming a pair of small shallow grooves, as shown in text-fig. 7. Laterally these grooves are not clearly marked off from the general ependyma, which, as is well known, is much more strongly developed in the fœtus than in the adult, but is distinguished from the epithelium of the grooves by the much more superficially-placed nuclei. Mesially the edges of the grooves become more sharply defined (text-fig. 7), the ependyma having practically disappeared from beneath the commissure between the grooves (compare cat, text-fig. 5).

Towards the hinder end of the posterior commissure these two grooves unite again and become invaginated into the roof of the *iter* to form a mesocœlic recess exactly comparable to that already described in the chimpanzee, but more widely open below (text-fig. 8).

This recess is lined throughout by the characteristic ependyma of the sub-commissural organ, and tunnels forwards into the brain tissue above the posterior commissure for a distance of about 0·35 mm. Its full length is rather greater than this owing to the tube becoming bent back upon itself for about 0·06 mm. to its blind termination.

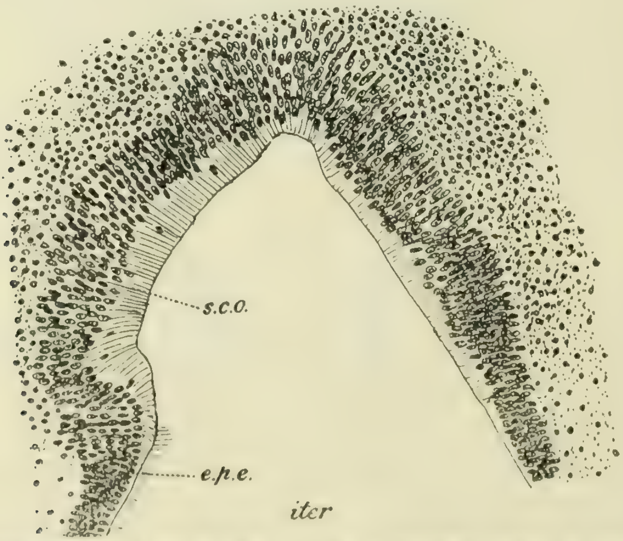

Fig. 8.—Human fœtus of about five months. Transverse Section through the Sub-Commissural Organ at the Posterior End of the Posterior Commissure. × 154.
e.p. e., ependymal epithelium of the *iter* ; *s.c. o.*, sub-commissural organ.

The diameter of the recess, as seen in text-fig. 9, is only about 0·02 mm., the recess being in this case much longer than wide—in fact, distinctly tubular. The lumen of the tube is somewhat triangular in transverse section (text-fig. 9), widening suddenly to its opening behind (text-fig. 8).

The elongated, radially arranged cells lining the recess are about 0·02 mm. in length.

Behind the recess the ependyma of the sub-commissural organ passes gradually into the general ependyma of the *iter*.

In sections through the region of the posterior commissure in the brain of the adult human subject the sub-commissural organ is seen to have almost entirely vanished. The ependyma covering the ventral surface of the commissure consists apparently of somewhat flattened cells with conspicuous nuclei, and differs very little from the general lining epithelium of the rest of the *iter*; the only difference being that upon the side walls of the *iter* the nuclei are more crowded, and possibly the epithelium slightly more cubic or columnar, but much less distinctly so than in the fœtus. The ependyma beneath the posterior commissure is continuous round the anterior surface of the latter with that which lines the infra-pineal recess, and in no wise differs from it. There is thus upon the surface of the commissure nowhere any indication of that characteristic elongatedly columnar epithelium that marks the sub-commissural organ in lower types.

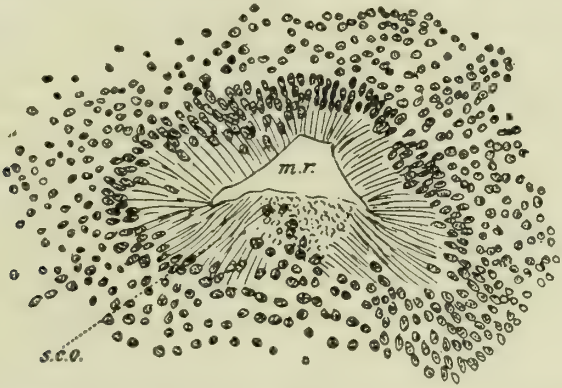

Fig. 9.—Human fœtus of about five months. Transverse Section through the Mesocœlic Recess, slightly anterior to the section represented in fig. 8. × 320.

m. r., mesocœlic recess; *s.c. o.*, sub-commissural organ, forming the epithelial lining of the mesocœlic recess.

At the hinder limit of the posterior commissure, however, about the middle line, there is an irregular thickening of the ependymal epithelium, and above this an irregular cavity (Plate 14, fig. 2, m.r.) occurs in the tissue of the brain, lined unevenly by the remains of an epithelium, with numerous scattered nuclei, exactly like that on the roof of the *iter* beneath it. No opening could be found putting this cavity into communication with the cavity of the *iter*, but numerous scattered nuclei connect the two epithelia, and suggest the remains of such a communication.

In successive sections this irregular cavity can be traced forwards and upwards, behind and above the posterior commissure. It obviously corresponds to the similarly situated mesocœlic recess in the fœtus, although lacking its characteristic high columnar ependymal lining. Anteriorly it again becomes choked with scattered cells, forming an ill-defined tract which passes forwards and then abruptly gives place to a small, but very well defined, sub-spherical chamber (Plate 14, fig. 3, m.r.). This chamber, moreover, is lined by exactly the same type of elongated epithelium as characterises the sub-commissural organ in the fœtus and in lower forms. The photomicrograph reproduced in Plate 14, fig. 3, shows this chamber, which we regard as simply the blind anterior termination of the mesocœlic recess (compare Plate 14, fig. 1, and text-fig. 9). The lumen here has a diameter of about 0·05 mm., while the cells of the lining epithelium are about 0·02 mm. long, with the nuclei placed as usual towards their inner ends. In the lumen is a small quantity of coagulum.

In the brain tissue around the posterior portion of the mesocœlic recess (*i.e.* that portion where the cavity has become irregular and largely obliterated) occur numerous small globules (Plate 14, fig. 2, gl.) which have stained brightly red in the preparations (which were treated with borax carmine in bulk, followed, upon the slide, by picro-indigo-carmine). Whether these are due to pathological or to *post-mortem* changes we are unable to say; that they are normally present seems improbable—in any case they are conspicuous in these sections, and interesting as occurring closely adjacent to the last vestiges of the sub-commissural organ.

We have thus found the sub-commissural organ fairly well developed in the human fœtus, and in an entirely vestigial condition in the adult. We have, however, only been able to examine one series of sections in each case. Probably, like many other vestigial structures, it will be found to vary considerably in the degree to which it persists in different individuals.

IV. *The Function of the Sub-Commissural Organ and Reissner's Fibre.*

Sargent, who has done so much to extend our knowledge of Reissner's fibre, considered (1904) this structure to be of a nervous nature, forming a kind of short circuit for optic motor reflexes. Our own histological investigations on many types do not support this view, nor do the physiological experiments of Sir Victor Horsley and Dr. McNalty (1908). On the other hand, there is every reason to believe that Reissner's fibre is a highly elastic structure, for it is well known that it has a strong tendency, when cut across, to spring back and coil itself into knots and spirals.*

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* *Cf.* Nicholls (1909).

Moreover, one of us (Nicholls) has been able recently to demonstrate beyond question that at its posterior end (in the lamprey and various teleosts) the fibre is not connected with "canal cells" in the *canalis centralis* of the spinal cord, as maintained by Sargent, but is connected through a terminal foramen in the neural tube with the surrounding connective tissue. Thus Studnička's statements on the subject (1899), controverted by Sargent (1904), are confirmed. At its anterior end the fibre appears always to break up into very slender branches, which are connected with the epithelial cells of the sub-commissural organ.

Reissner's fibre and the sub-commissural organ occur throughout the vertebrate series from the cyclostomes to the primates, and there can be little doubt that where they are (as in nearly all cases) well developed they must have some important function.

In a letter recently published in 'Nature' one of us (Dendy, 1909) has made a suggestion as to the possible function of these organs, which appears to us to be strongly supported by the results recorded in the present communication. This suggestion was to the effect that Reissner's fibre and the sub-commissural organ ("Ependymal Groove") may form part of an apparatus for regulating flexure of the body. It was pointed out that any such flexure would tend to alter the tension of Reissner's fibre, and thereby exert a mechanical stimulus upon the epithelial cells of the sub-commissural organ to which it is attached anteriorly. It was supposed that the stimulus thus received by the sensory epithelial cells might be transmitted to appropriate nerve-cells in the brain, and that the deviations of the long axis of the body from the normal position might thus be regulated by reflex action; and a comparison was made with the function of the semicircular canals of vertebrates, and also with that of the "Statocysts" of many invertebrates, which serve by means of mechanical stimuli, due in this case to the action of gravity, automatically to regulate the orientation of the body.

The fact that man, almost the only vertebrate which has assumed the erect posture, and one of the few which have completely lost the tail, also has the sub-commissural organ in a more reduced condition than in any other case known, while the semi-erect and almost tailless chimpanzee is in this respect in an intermediate condition between man and the lower vertebrates, seems clearly to indicate that the function of the sub-commissural organ is in some way connected with the position of the body and the flexibility of the vertebral column (including of course the tail).

Considering that the sub-commissural organ, with which Reissner's fibre, when present, is invariably connected, is reduced to a mere vestige in the adult man, it seems extremely improbable that man possesses any Reissner's fibre at all. The only primates in which the presence of Reissner's fibre has yet been demonstrated are *Macacus cynomolgus* and *M. rhesus* (Horsley, 1908), and it is perhaps significant that these are both tailed and non-erect forms.

We may perhaps point out that the suggestion thus put forward as to the possible function of Reissner's fibre and the sub-commissural organ is not inconsistent with the view previously maintained by one of us (Dendy, 1902), to the effect that in the Ammocœte the latter may serve by ciliary action to promote the circulation of the cerebro-spinal fluid.

We should like to express our indebtedness to our colleagues Prof. Waterston, Prof. Peter Thompson, and Mr. Frazer, for placing at our disposal some of the material upon which this paper is based, and Mr. R. W. H. Row for making the photomicrographs used in illustration.

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DESCRIPTION OF PLATE 14.

Fig. 1.—Chimpanzee. Photomicrograph of part of a Transverse Section through the Posterior End of the Posterior Commissure, showing the Sub-commissural Organ and the Mesocœlic Recess. × 34.

Fig. 2.—Man (adult). Photomicrograph of part of a Transverse Section through the Posterior End of the Posterior Commissure, showing the Irregular Proximal (posterior) part of the Mesocœlic Recess. × 58.

Fig. 3.—Man (adult). Photomicrograph of part of a Transverse Section through the Posterior Part of the Posterior Commissure at a level slightly anterior to that of the section represented in fig. 2, to show the terminal (anterior) portion of the mesocœlic recess, with its characteristic columnar epithelium. × 300.

EXPLANATION OF LETTERING.

b. v., blood vessels ; gl., globular bodies ; m. r., mesocœlic recess ; p. c., posterior commissure ; s.c. o., sub-commissural organ.

Autotoxæmia and Infection.

By E. C. Hort, F.R.C.P. Ed., Assistant Physician to the Italian Hospital.

(Communicated by William Osler, F.R.S. Received June 2,—
Read June 23, 1910.)

The object of this communication is to show :—

That fever,* loss of weight, and a rise in the antitryptic values of the blood serum, three results common to infection in man, can be reproduced in animals by the subcutaneous injection of small quantities of distilled water.

CONTROL OBSERVATIONS.

The following precautions were found to be necessary in order to demonstrate these effects, apart from selection of healthy animals for injection, and observance of strict aseptic conditions as to site of injection, instruments used, and water injected :—

Fever.

1. Selection and repeated testing of thermometers of guaranteed accuracy.
2. Accurate approximation of the temperature of the water injected to the temperature of the animal receiving the injection.
3. Determination of the mean average, and of the upper normal limit of, temperature of the species of animal selected for injection.
4. Demonstration that repeated handling and the taking of repeated thermometric observations do not cause a rise of temperature in the control animals.
5. Demonstration that mere subcutaneous puncture, or injection of air equivalent in bulk to the volumes of water injected, does not cause fever in the control animals.

---

* By fever is here meant a rise of temperature, and nothing more.

Loss of Weight.

6. Selection of a species of animal, such as the guinea-pig, on which reliable observations as to weight can be made for several days previous and subsequent to injection in order to establish necessary control.

7. Exclusion of error owing to any loss of weight being due to refusal of the animals to take food after injection.

Rise in Antitryptic Values of Serum.

8. Demonstration of the accuracy of the method used to measure antitryptic values.

9. Demonstration of strict parallelism between the antitryptic values of the serums of normal individuals of the species of animal injected.

The precautions requiring further detail are those numbered 3, 4, 5, 7, 8, 9.

3. The mean average temperature of 135 guinea-pigs selected for experiment was 101·4° F., or 38·6° C., the upper normal limit of temperature being 102·2° F., or 39° C. The mean average temperature of 50 apparently healthy rabbits was 102·4° F., or 39·1° C., the upper normal limit of 103° F., or 38·6° C., usually assigned, being accepted.

4. Repeated handling *per se* was invariably found to produce no rise of temperature in healthy guinea-pigs or rabbits. Unless, however, great gentleness be employed in taking repeated thermometric observations on the guinea-pig, fever is easily induced, owing to slight abrasion of rectal mucous membrane caused by insertion of the thermometer and consequent septic absorption. This does not occur in the rabbit with ordinary care. Hence the effect of multiple injections involving a series of thermometric observations extending over several days can only be satisfactorily studied in the rabbit. This does not apply to the study of the effects of single injections in the guinea-pig, as all observations can be completed in one day. After injury to rectal mucous membrane there is always a latent period of not less than 12 hours before fever caused by introduction of the thermometer appears, whereas the fever caused by injection of water is never more than five hours in reaching its maximum after injection. Hence in animals controlling injections on the first day a rise of temperature is always absent, provided that the animal is healthy.

5. Neither subcutaneous puncture nor injections of air up to 10 c.c., the maximum quantity of water injected into guinea-pigs in these experiments for the demonstration of fever, cause any rise of temperature in guinea-pigs.

7. The loss of weight observed in guinea-pigs after multiple injections of water can be shown not to be due to refusal of the animals to take food,

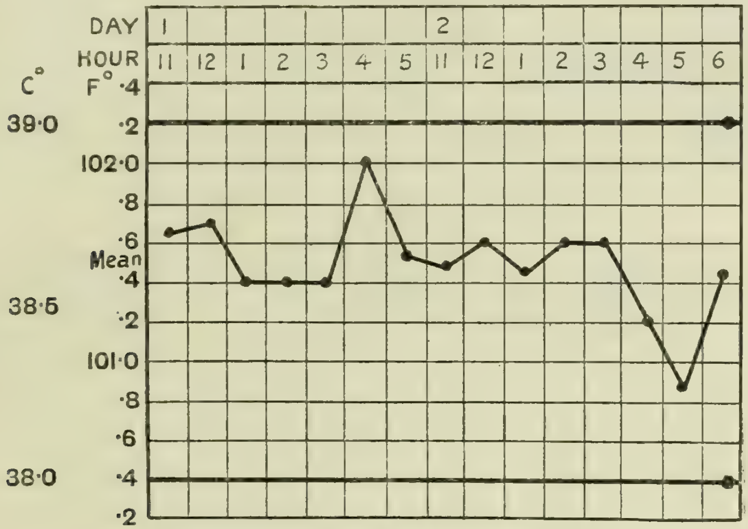
if they are kept in separate cages, and if the amount of food supplied and devoured be carefully noted.

8. *Vide* heading below, "Antitryptic values."

9. *Vide* heading below, "Antitryptic values."

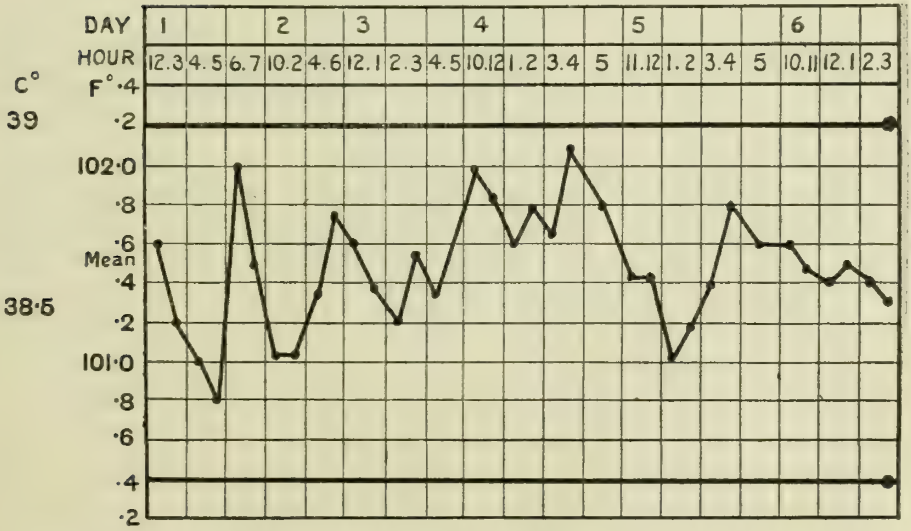
In order to represent in a graphic form the necessary control observations, the actual charts are subjoined showing absence of fever in uninjected animals.

CHART 1.—Guinea-pig. (Control.)



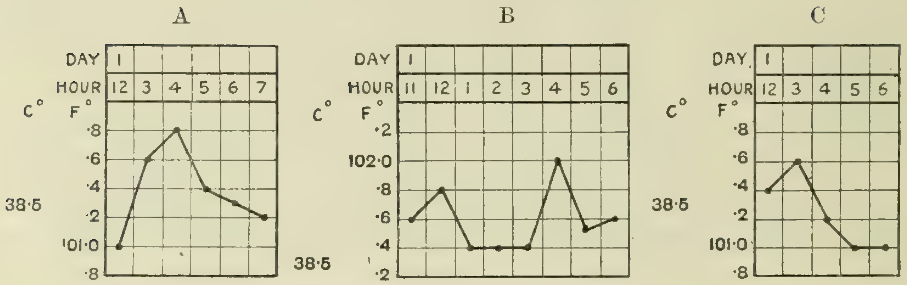
Upper normal limit = 102·2° F., 39° C.

CHART 2.—Guinea-pig. (Control.)

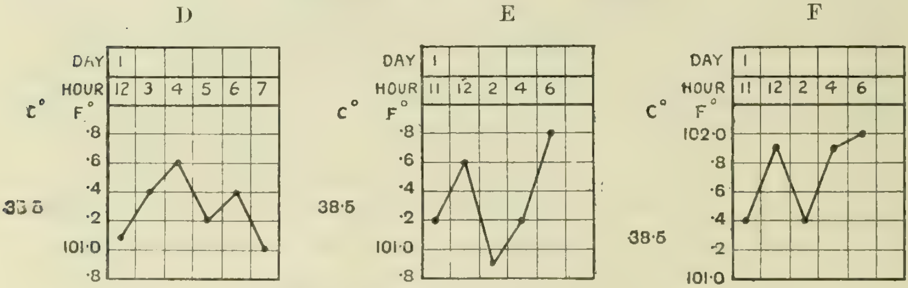


Upper normal limit = 102·2° F., 39° C.

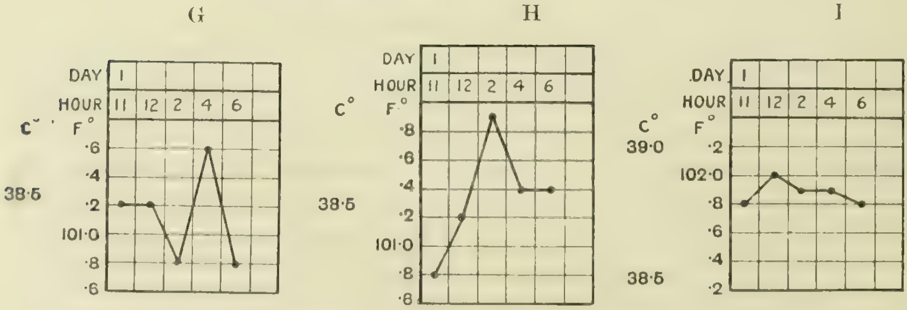
Chart 3.—Guinea-pigs. (Controls.)



Normal upper limit = 102·2° F., 39° C.

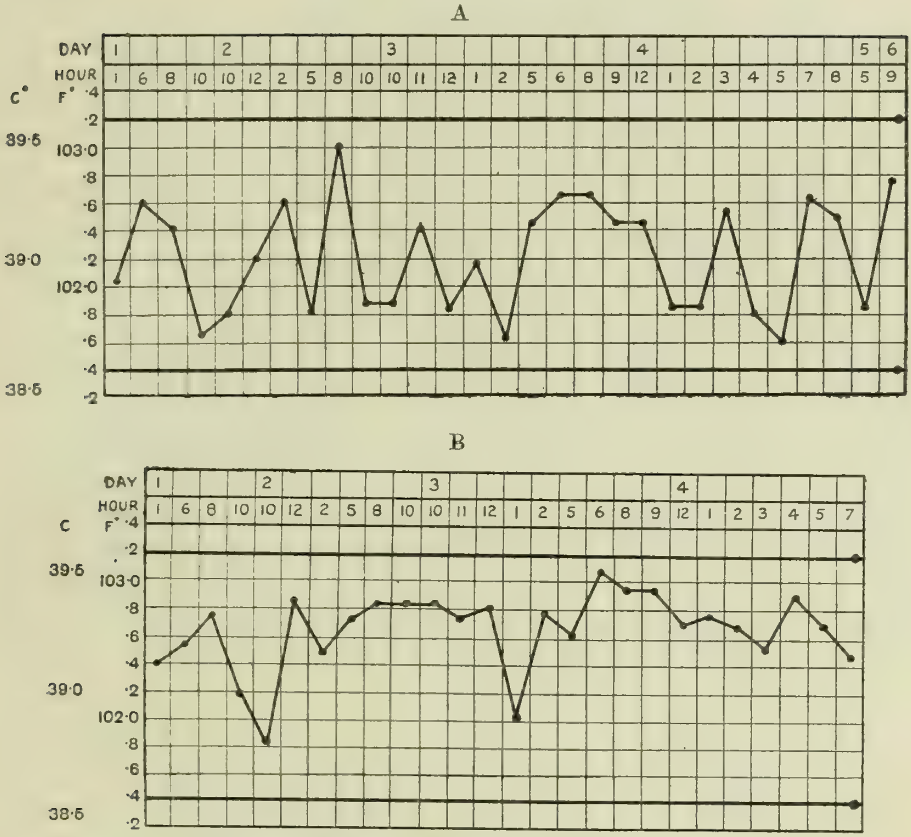


Normal upper limit = 102·2° F., 39° C.



Normal upper limit = 102·2° F., 39° C.
Mean = 101·5° F., 38·6° C.

CHART 4.—Rabbit. (Control.)



EXPERIMENTS.

The results of injection of water were as follows :—

A. *On Temperature.*

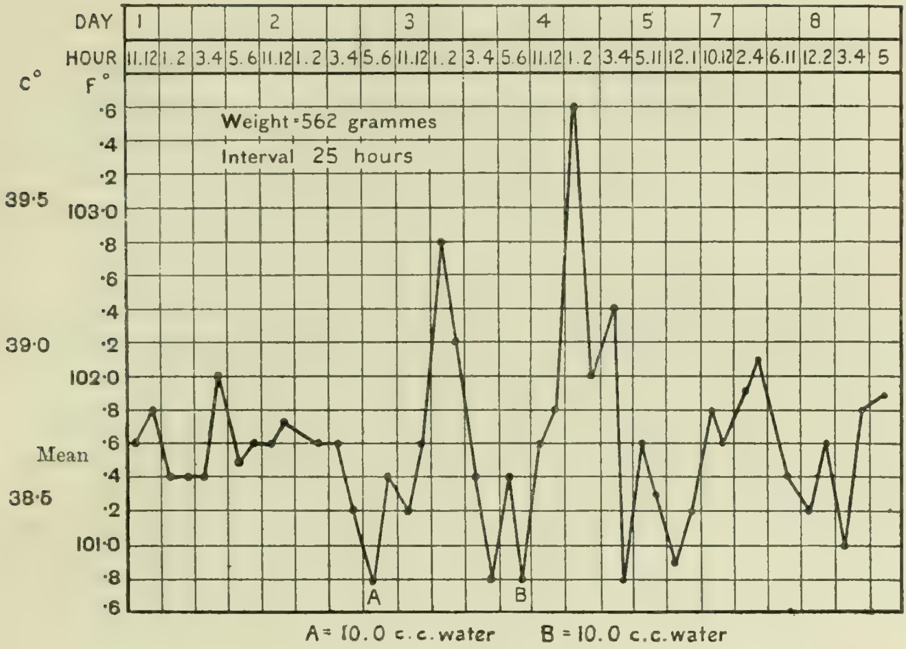
1. *Single Injections.*—Sixty guinea-pigs, varying in weight from 100 to 600 grammes, were injected under the skin of the abdomen with single injections of boiled distilled water, in quantities varying from 1 c.c. to 10 c.c. A rise of temperature was observed in 50. It has so far not been possible to establish a constant between the bulk of injection, the weight of the animal injected, and the degree of fever produced. A marked rise has occurred after injection of a quantity of water equivalent to 1/304 of the body weight. The rise in temperature after single injections is rapid and fugitive. It attains its maximum in from 2 to 5 hours. A rise above 103° F. or 39·5° C. after first injections is exceptional, and is often less. The rise is easily missed unless repeated observations are taken, at least once an hour. Twelve rabbits

received subcutaneous injections of water in quantities varying from 10 to 60 c.c. With the former quantity a slight rise occurs, even in rabbits weighing $2\frac{1}{2}$ kilogrammes, with the latter a marked rise, sometimes to 104·5° F. or 40·3° C. Some elevation of temperature above the normal occurred in all 12 animals. An injection of 23 c.c. into a marginal vein of the ear of a rabbit weighing $1\frac{1}{2}$ kilogramme caused a rise to 106° F., or 41·1° C. The rise in rabbits shows roughly the same abruptness, the same length of time to reach the maximum, and the same fugitive nature as in guinea-pigs.

2. *Multiple Injections.*—The effect of multiple injections in both rabbit and guinea-pig is to cause a continuous fever lasting only so long as the injections are continued, abruptly ceasing as soon as the injections are stopped. Multiple injections were given to 30 guinea-pigs and 10 rabbits, and caused fever in every case. To several other guinea-pigs than these 30 injections larger than 10 c.c. were given, and instead of a rise of temperature a profound fall followed. The same effect can be produced by giving small injections, closely spaced. On correct spacing of two injections into the same animal hypersensitisation is frequently induced, within limits not yet determined, but clearly evidenced by the rise of temperature induced by the second injection being greater than that induced by the first. Owing to the difficulty already mentioned of repeated thermometric observations extending over several days in the guinea-pig, study of the effect of multiple injections of more than two in number is unsatisfactory, but the effects of hypersensitisation in this animal after two injections are convincing. For study of the effects of injections over a series of days the rabbit must be used.

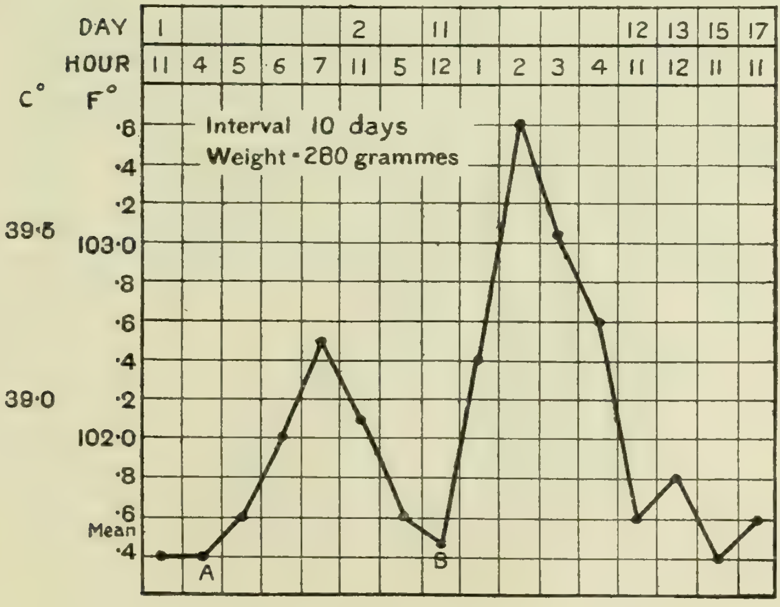
It is impossible to reproduce all the very numerous charts illustrating the induction of fever after both single and multiple injection in the rabbit and guinea-pig, but a few typical charts are subjoined for comparison with the control charts given above.

Chart 5.—Guinea-pig.



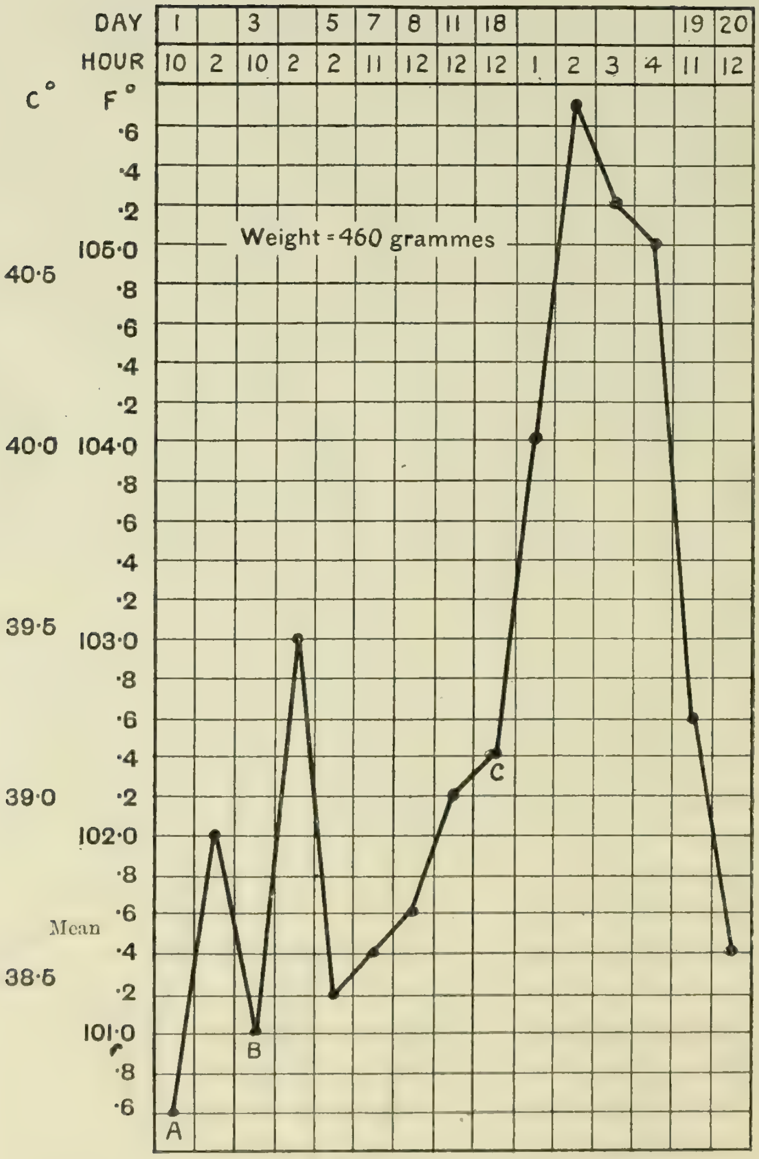
A = 10.0 c.c. water B = 10.0 c.c. water

Chart 6.—Guinea-pig.



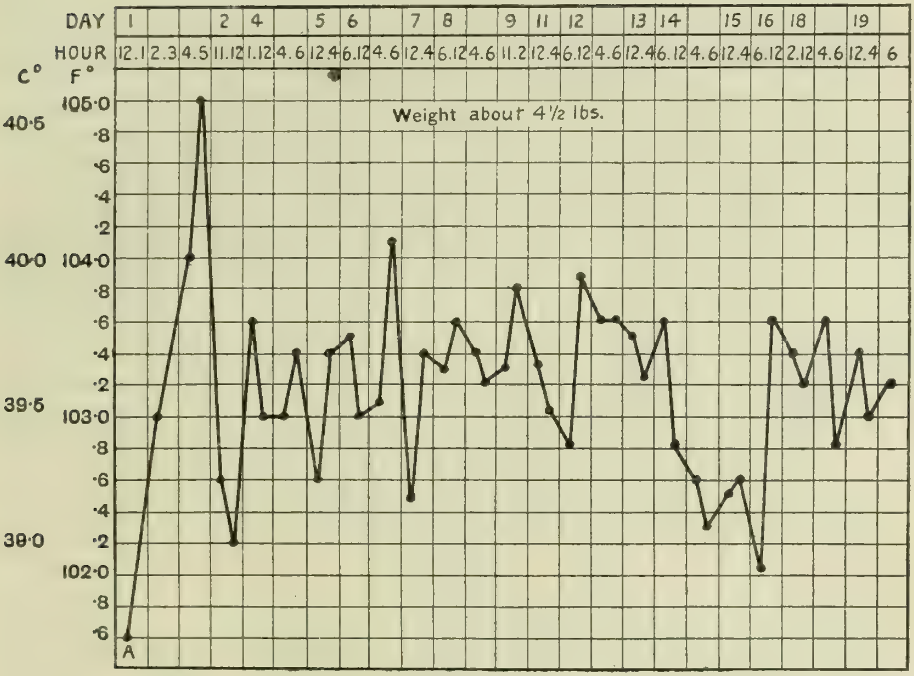
A = 5.0 c.c. water B = 5.0 c.c. water

Chart 7.—Guinea-pig.



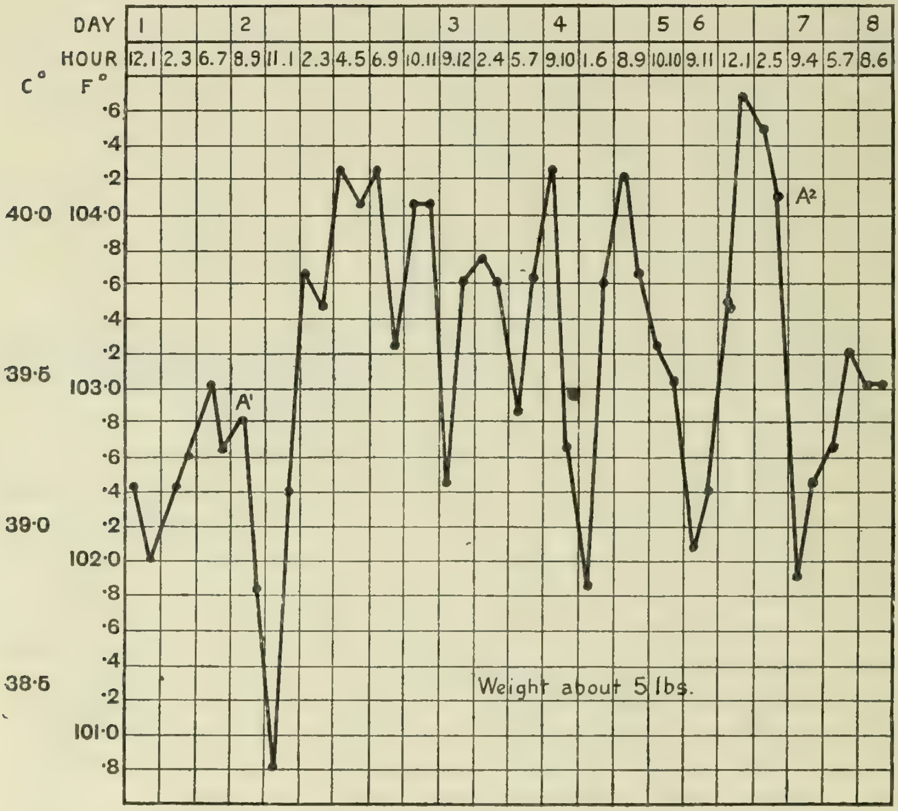
A.B.C.= 5.0 c.c. water

Chart 8.—Rabbit.



A= 50. 0 c.c. water

Chart 9.—Rabbit.



A^1 = first injection, 40 c.c. Twelve injections in all, with a total bulk of 246 c.c. from A^1 to A^2.

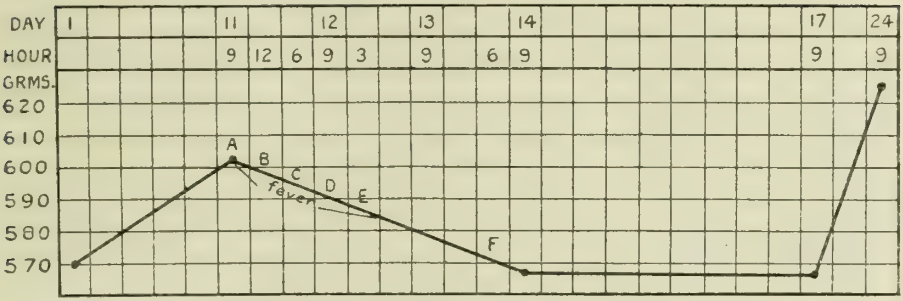
B. *On Body Weight.*

Observations on variations in the weight after single small injections of water into the guinea-pig were inconclusive.

The results on weight of multiple injection are illustrated by the subjoined charts, which are self-explanatory.

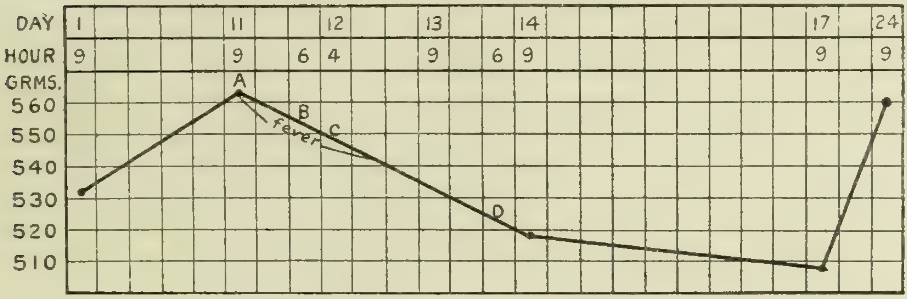
CHART 10.

A.



A. B. C, D E. F = 100.0 c.c. in divided doses in 33 hours

B.



A. B., C, D. = 70.0 c.c. in divided doses in 33 hours

C. *On the Antitryptic Values of the Serum.*

(a) *Rabbits.*

Twenty cubic centimetres of water were injected subcutaneously, in four divided doses, into a healthy rabbit. The animal was bled immediately before the first injection, and 60 minutes after the last injection, to the extent of 5 c.c. on both occasions. A control animal was bled at the same times, but was not injected. Estimation of the antitryptic values of all four serums was made the following morning. In order to ensure accuracy of observation in estimation of the antitryptic values of the serums two methods were employed: (1) The chemical method of Sörensen[1] for estimating the velocity of action of a trypsin-casein mixture (by an adaptation of which I had shown that it was possible[2] to determine variations in the antitryptic values of the serum of subjects of malignant disease with sufficient accuracy to aid materially in diagnosis). (2) The viscosity method introduced by Spriggs for estimating the velocity of a trypsin digestion adapted by Golla[3] for determining antitryptic values of serum in normal and pathological conditions. The results given by both methods in determining the values of the serums after injection of water were parallel and mutually confirmatory. The results of the viscosity method, slightly modified by myself, being the simplest, are given below. To 10 c.c. of a 40-per-cent. mixture of casein and water is added 0·25 c.c. of the serum of the antitryptic value of which estimation is desired. At a fixed time 1 c.c. of a 1-per-cent. filtered fresh solution of trypsin in water is added to the casein mixture, and digestion allowed to proceed at a constant temperature, in this case 19° C. Before adding the trypsin solution, observations are taken with a stop-watch to determine the length of time occupied by the discharge from the viscosimeter of the contents of the bulb between two marked points. After addition of the trypsin solution, repeated observations are taken at intervals in exactly the same way. All the serum-casein-trypsin mixtures are then put through the viscosimeter at the same intervals of time, and in this way comparable results are obtained.

Experiment.

	Injected rabbit before first injection.	Injected rabbit after last injection.	Control rabbit before time of first injection.	Control rabbit after time of last injection.
Discharge time before addition of trypsin	2.49	2.49$\frac{1}{5}$	2.49$\frac{1}{5}$	2.49
First reading, 16 minutes after addition of trypsin	2.12	2.12$\frac{3}{5}$	2.12	2.11$\frac{3}{5}$
Second reading, 45 minutes after addition of trypsin	2.0	2.1$\frac{1}{5}$	2.0$\frac{1}{5}$	1.59$\frac{3}{5}$
Third reading, 110 minutes after addition of trypsin	1.50$\frac{1}{5}$	1.51$\frac{4}{5}$	1.50$\frac{1}{5}$	1.50

From these figures it is clear that, whereas, in the case of the control, the serum from the second bleeding showed, if anything, a somewhat lower antitryptic value than the serum from the first bleeding, the serum of the injected animal showed after injection a slight but unmistakable rise in antitryptic values compared with the reading before injection, in spite of the smallness of the injection. The weight of the animal was almost 5 lbs.

(b) *Guinea-pigs.*

A guinea-pig, weighing 442 grammes, received subcutaneously an injection of 5 c.c. of water. This was followed in three hours by a rise of temperature, the height of which was intensified by a previous injection of the same bulk 16 days previously, and by a third injection of the same bulk 18 days previously. The final injection was followed by a fall of weight from 442 grammes to

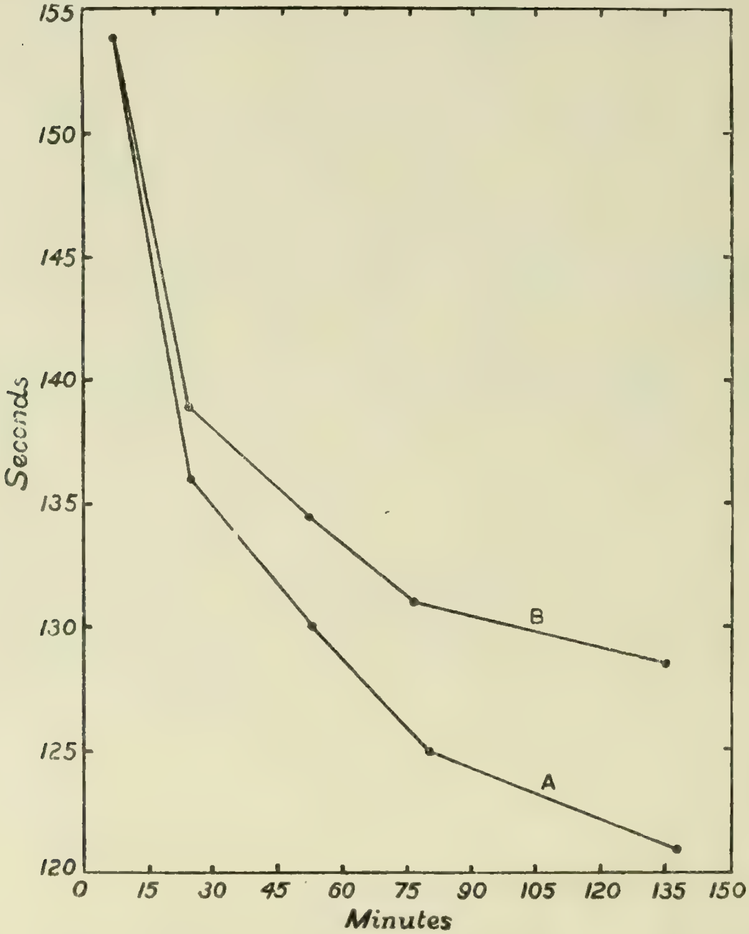
429 grammes. Four days after the injection the animal was killed and estimation made of the antitryptic values of the serum. The estimations were made in duplicate. Temperature, 18° C. throughout. Quantities as before.

Experiment.

	Control 1A.	Control 1B.	Injected animal 2A.	Injected animal 2B.
Discharge time before addition of trypsin	3. 19$\frac{3}{5}$	3. 19$\frac{2}{5}$	3. 20	3. 20
First reading, 6 minutes after addition of trypsin	2. 34$\frac{2}{5}$	2. 34$\frac{3}{5}$	2. 34$\frac{3}{5}$	2. 34$\frac{4}{5}$
Second reading, 35 minutes after addition of trypsin	2. 16	2. 16$\frac{2}{5}$	2. 19	2. 18$\frac{2}{5}$
Third reading, 65 minutes after addition of trypsin	2. 10	2. 10$\frac{2}{5}$	2. 15	2. 14$\frac{4}{5}$
Fourth reading, 95 minutes after addition of trypsin	2. 5$\frac{1}{5}$	2. 5$\frac{2}{5}$	2. 11$\frac{3}{5}$	2. 11$\frac{2}{5}$
Fifth reading, 155 minutes after addition of trypsin	2. 2	2. 2$\frac{1}{5}$	2. 8$\frac{2}{5}$	2. 8$\frac{1}{5}$

These results may be expressed in the form of a curve (p. 542), from which it will be seen that in this case the effect of multiple injections of water was to give rise to a high antitryptic value in the serum of the injected animal, represented at the maximum point of divergence by approximately six seconds.

Viscosity Curve showing Antitryptic Power of Serum after Injection of Water. All four serums at same point six minutes after commencement of digestion.



The validity of these last figures rests on demonstration that the serums of healthy animals of the same species give closely parallel antitryptic values. This I have been able to show in the case of the horse, rabbit, and guinea-pig, by numerous observations as yet unpublished. This has already been shown for man.[4] The serums of two healthy guinea-pigs, taken at random, gave the following readings. The estimations were done in duplicate. Temperature, 16° C. throughout. Quantities as before :—

	Animal 1A.	Animal 1B.	Animal 2A.	Animal 2B.
Discharge time before addition of trypsin	$3.15\frac{3}{5}$	3·15	$3.16\frac{2}{5}$	$3.15\frac{4}{5}$
First reading, 6 minutes after addition of trypsin	$2.50\frac{4}{5}$	$2.51\frac{1}{5}$	$2.51\frac{2}{5}$	$2.51\frac{3}{5}$
Second reading, 35 minutes after addition of trypsin	2. 39	2. 39	$2.39\frac{3}{5}$	2. 39
Third reading, 65 minutes after addition of trypsin	$2.30\frac{2}{5}$	$2.30\frac{2}{5}$	$2.30\frac{4}{5}$	$2.30\frac{4}{5}$
Fourth reading, 95 minutes after addition of trypsin	$2.27\frac{1}{5}$	2. 27	$2.27\frac{3}{5}$	$2.27\frac{2}{5}$

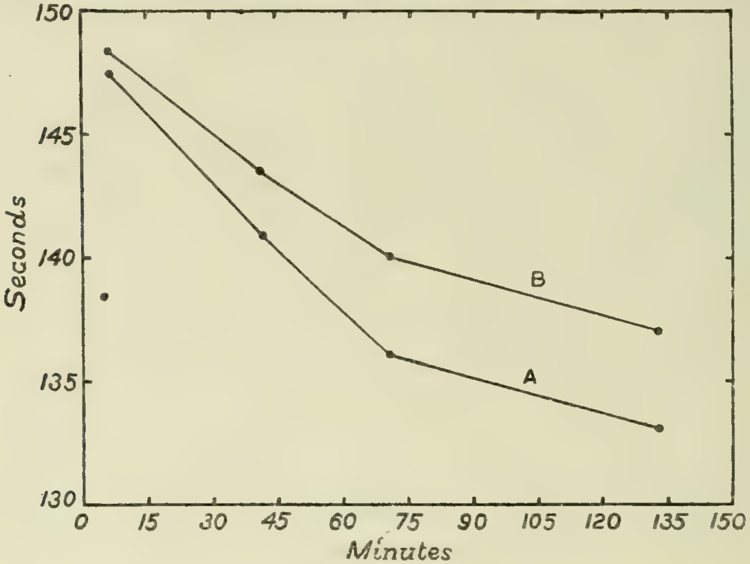
The difference between the means of the two duplicates of the two serums is not in excess of three-fifths of a second, once digestion has begun.

Comparison of the following curve (p. 544) with the preceding curve illustrates the gross resemblance that exists between the curves of animals that have received injections of water only, and of those that have been artificially infected with bacterial products.

A guinea-pig of standard weight was injected on Saturday, October 23, 1909, at 10 A.M., with 0·91 of a M.L.D. of diphtheria toxin. On the following Monday, at 10 A.M., he was killed, and the antitryptic value of the serum estimated the following day. Estimations in duplicate. Temperature, 12° C. throughout.

	Control 1A.	Control 1B.	Injected animal 2A.	Injected animal 2B.
First reading, 15 minutes after addition of trypsin	$2.28\frac{3}{5}$	$2.28\frac{2}{5}$	$2.29\frac{4}{5}$	$2.29\frac{1}{5}$
Second reading, 45 minutes after addition of trypsin	$2.21\frac{1}{5}$	$2.21\frac{2}{5}$	$2.24\frac{3}{5}$	$2.24\frac{1}{5}$
Third reading, 75 minutes after addition of trypsin	$2.16\frac{3}{5}$	$2.16\frac{4}{5}$	$2.20\frac{2}{5}$	2. 20
Fourth reading, 135 minutes after addition of trypsin	2. 13	$2.13\frac{1}{5}$	$2.17\frac{2}{5}$	2. 17

Viscosity Curve showing Antitryptic Power of Serum after Injection of Diphtheria Toxin.



A, Serum of control animal, mean of duplicates.
B, Serum of injected animal, mean of duplicates.

Conclusions.

These experiments show that, as regards gross results, there is a close parallelism between :—

(1) The temperature charts of animals that have received injections of water and of those that have received injections of living bacteria, or of solutions of bacterial toxins.

(2) The weight curves of animals that have received multiple injections of water and of those that have received injections of living bacteria, or of solutions of bacterial toxins.

(3) The antitryptic values of the serums of animals that have received multiple injections of distilled water, or of solutions of bacterial toxins.

They lend strong support, in fact, to the view I have already advanced,[5] based on clinical observation, that however great the share taken by micro-organisms, including protozoa, and their products in initiating the disease complex of infection, the net result is, perhaps to a large extent, a state of true auto-intoxication.

Note.—The only observations on the effect of subcutaneous injection of small quantities of distilled water as regards the production of fever that I can find in the literature are those of Krehl in 1897, who specifically denies, as a result of his observations, that the injection of distilled water by the subcutaneous route can cause fever.[6] In the paper referred to it is clear that neglect to make hourly observation after injection is responsible for his statement.

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The Blood Volume of Mammals as Determined by Experiments upon Rabbits, Guinea-pigs, and Mice, and its Relationship to the Body Weight and to the Surface Area expressed in a Formula.

By GEORGES DREYER, M.A., M.D., and WILLIAM RAY, B.Sc., M.B.

(Communicated by Prof. F. Gotch, F.R.S. Received May 5,—Read June 23, 1910.)

(Abstract.)

The blood volume of animals has for many years been the subject of numerous investigations. This is but natural, considering its great importance for the study of disease. As, however, the results obtained are very discordant, we have determined the blood volumes of rabbits, guinea-pigs, and mice by Welcker's method, by washing out the circulatory system, and by following the percentage fall of hæmoglobin after bleeding.

Our experiments have given the following results:—

(1) The blood volume of living mammals can be determined very accurately by bleeding the animal (about 20 per cent. of its original blood volume) and determining the percentage fall of hæmoglobin at the moment when equilibrium is reached. This method gives results remarkably concordant with those obtained by washing out the circulatory system. In employing this method it is absolutely essential that the animals should not have been bled before.

(2) In normal healthy mammals (rabbit, guinea-pig, and mouse) the blood volume is satisfactorily expressed by the following formula, $B = W^{\frac{2}{3}}/k$, where B is the blood volume in cubic centimetres, W the weight of the individual in grammes, and k a constant to be ascertained for each particular species of animal. This formula indicates that the smaller animals of any given species, which have a relatively greater body surface than heavier ones, have also a relatively greater blood volume. That is to say, that the blood volume can be expressed as a function of the surface area. It is therefore misleading to express the blood volume as percentage of the body weight, as has hitherto been invariably done.

(3) The constant k, by means of which the blood volume in cubic centimetres can be calculated from our formula $B = W^{\frac{2}{3}}/k$ when the weight of the animal in grammes is known, is approximately, for—

Rabbit	1·58
Guinea-pig............ ..	3·30
Mouse....................	6·70

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Page

THE EFFECT OF PRESSURE UPON ARC SPECTRA. No. 3.—SILVER; No. 4.—GOLD. By W. G. DUFFIELD, D.Sc. (Abstract) .. 118

STUDIES OF THE PROCESSES OPERATIVE IN SOLUTIONS. XI.—THE DISPLACEMENT OF SALTS FROM SOLUTION BY VARIOUS PRECIPITANTS. By H. E. ARMSTRONG, F.R.S., and J. VARGAS EYRE, Ph.D. .. 123

THE DISTRIBUTION OF VELOCITY IN THE β-RAYS FROM A RADIO-ACTIVE SUBSTANCE. By J. A. GRAY, B.Sc., 1851 Exhibition Scholar, University of Melbourne. (Plate 1) .. 136

THE DECREASE OF VELOCITY OF THE β-PARTICLES ON PASSING THROUGH MATTER. By WILLIAM WILSON, M.Sc., Hon. Research Fellow of the University of Manchester .. 141

RATE OF EMISSION OF α-PARTICLES FROM URANIUM AND ITS PRODUCTS. By J. N. BROWN, A.R.C.S. .. 151

ON RADIATION IN A GASEOUS EXPLOSION. By BERTRAM HOPKINSON, F.R.S. 155

THE EFFECT OF SMALL TRACES OF WATER VAPOUR ON THE VELOCITIES OF IONS PRODUCED BY RÖNTGEN RAYS IN AIR. By ROBERT TABOR LATTEY.. 173

ON THE VARIATION WITH TEMPERATURE OF THE VISCOSITIES OF THE GASES OF THE ARGON GROUP. By A. O. RANKINE, D.Sc., Assistant in the Department of Physics, University College, London .. 181

ADDITIONAL REFRACTIVE INDICES OF QUARTZ, VITREOUS SILICA, CALCITE, AND FLUORITE. By J. W. GIFFORD .. 193

THE ACCUMULATION OF HELIUM IN GEOLOGICAL TIME. IV. By the Hon. R. J. STRUTT, F.R.S., Professor of Physics, Imperial College of Science, South Kensington .. 194

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Series B. Vol. 82. No. **B 559.**

BIOLOGICAL SCIENCES.

CONTENTS.

Page
CROONIAN LECTURE.—Alterations in the Development and Forms of Plants as a Result of Environment. By Prof. G. KLEBS, University of Heidelberg 547

The Origin and Destiny of Cholesterol in the Animal Organism. Part VII.—On the Quantity of Cholesterol and Cholesterol Esters in the Blood of Rabbits Fed on Diets containing Varying Amounts of Cholesterol. By MARY T. FRASER, B.Sc., and J. A. GARDNER, M.A. 559

On the Comparative Toxicity of Theobromine and Caffeine, as Measured by their Direct Effect upon the Contractility of Isolated Muscle. By V. H. VELEY, F.R.S., and A. D. WALLER, M.D., F.R.S. 568

A New Method for the Quantitative Estimation of Hydrocyanic Acid in Vegetable and Animal Tissues. By Dr. A. D. WALLER, F.R.S. . . 574

The Origin of Osmotic Effects. III.—The Function of Hormones in Stimulating Enzymic Change in Relation to Narcosis and the Phenomena of Degenerative and Regenerative Change in Living Structures. By HENRY E. ARMSTRONG, F.R.S., and E. FRANKLAND ARMSTRONG, Ph.D., D.Sc. 588

The Inorganic Composition of the Blood in Vertebrates and Invertebrates, and its Origin. By Prof. A. B. MACALLUM, Ph.D., Sc.D., F.R.S., of the University of Toronto 602

The Anatomy and Morphology of the Leaves and Inflorescences of Welwitschia mirabilis. By Miss M. G. SYKES. (Abstract) . . . 625

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No. B 559.

September 9, 1910.

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CROONIAN LECTURE.—*Alterations in the Development and Forms of Plants as a Result of Environment.**

By Prof. G. KLEBS, University of Heidelberg.

(Lecture delivered May 26,—MS. received June 25, 1910.)

I have to-day the great honour of delivering an address before the Royal Society, so long renowned as a centre of natural science; but I do so with a certain hesitation, being aware that I am not sufficiently master of the English language, and have had but little time for preparation. In consideration of this, I shall avoid entering too much into theoretical discussion; I wish rather to lay stress upon the demonstration of the results of my experiments upon *the Influence of Environment on Plants.*

The problem of the relations of the organism to its surroundings is very old, but it has only recently become an object of active research. In all branches of biology, as well as in the different fields of history, educational science, and so forth, we meet this fundamental problem and also the most conflicting views on it, some of which suffer from exaggeration of the influence of environment, others from its under-estimation.

We naturalists are guided above all by experience, and we use theoretical opinions to select such questions as can be tested by experiments. In this way physiology tries to recognise the life-processes in their dependence on external factors. Nutrition, growth, and all phenomena associated with stimulus and movement have been studied with great success. On the other hand, the phenomena connected with the production of forms have been neglected, remaining exclusively a branch of the purely descriptive science of morphology. In modern times, however, physiology claims to penetrate this mysterious region by means of experiment.

One of the most striking features of an organism is its development from a fertilised egg to maturity. Every species goes through a clearly defined development, and shows from its first genesis a complicated series of successive changes of form, of which each is the necessary outcome of its predecessor. This exceedingly regular course of development, consisting of successive internal alterations, is preserved under different conditions of life.

We can understand the presumption, considered self-evident in former times, and having its supporters even now, that this development is the expression of the inscrutable inner nature of plants. According to this, external factors only supply material and energy to build up the organism, without influencing the course of its development. But if this presumption were really true, we should have to give up hope of attaining anything more than purely descriptive treatment of form. Fortunately, however, as modern biology shows, we can prove more and more that development can be altered in a far-reaching degree. The fact that the internal alterations causing the definite form can be modified by alterations of environment is the key which opens the door to research.

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* The Lecture as delivered was illustrated by numerous photographic slides, the publication of which is deferred for the present.

It has been known for a long time that water-plants, when brought into a relatively dry atmosphere, change their form, and numerous other examples of form-modification due to environment are familiar to us. But now we wish to elucidate the whole phenomenon of development, and to recognise the conditions of every stage. Our aim is to control the whole life of the plant.

It is obvious, however, that we can only realise that which potentially exists in the internal structure of the species. This structure, with all its potentialities, forms the basis of our experiments and can never be explained. But this idea of further potentialities is what guides us in our quest of new discoveries. The manner of development of a plant under ordinary conditions is only one of its many possibilities. Nature must be much richer than appears under the influence of ordinary environment. We seize upon this idea to create new kinds of environment, and thereby to produce new forms not otherwise occurring. We shall recognise the methods and aims of the physiology of development better by making ourselves acquainted with the facts than by long discussions.

I will first examine a single specimen of the lower plants—the fungus Saprolegnia—before we pass on to the higher ones. A white mould, which belongs to the fungoid genus *Saprolegnia*, and to the species *mixta*, appears very frequently upon dead insects in swamps. The fungus consists of tube-like branched filaments, known as the mycelium, which enter the body of the insect with their basal parts, and then spread out in the water. For the first few days, vegetative growth rules exclusively, whilst the filaments grow longer and branch. The second stage, characterised by the formation of asexual reproducing cells, follows the first. The ends of the filaments swell up a little and are separated by a partition, producing, by division, numerous very minute cells, known as zoospores. These make their exit through a hole and swim free in the water until they find a new substratum, in the shape of another dead insect, upon which they can settle. The formation of zoospores takes place again and again on the mycelium for some days.

Finally, a third stage of development follows, namely, the formation of sexual organs, male and female. Spherical cells are formed on the filaments, the so-called oogonia, which contain at least two and sometimes many more egg-cells, the oospheres. Upon other filaments branches arise, which grow into the oogonia and, pressing against them, produce the male antheridia. By means of a penetrating tube a part of the male fertilises the egg-cells, which turn into spores, germinating after some months of rest. The fungus itself dies after a few weeks of development.

The question is whether this very regular succession of the different stages, each with its special forms and functions, is really dependent on internal causes. If we want to recognise the conditions of this development, we must first know as exactly as possible the conditions of life, especially the manner of nutrition. The fungus is easy to cultivate either on a solid or in a liquid medium, which must contain principally nitrogenous substances, such as albumen, peptone, extract of peas, etc. In such very favourable solutions only vegetative growth of the mycelium takes place. When we take care to keep up the good nutrition by putting a piece of the mycelium into fresh food solution from time to time, it continues to grow without interruption, and can never reach any other stage of development, for instance, the asexual or the sexual reproduction. I devoted six years to carrying out this experiment.

We can just as little imagine that under constant external conditions the fungus reproduces by itself an alteration of its development, as that water becomes ice by itself under constant temperature and pressure. Therefore, under such constant environment the fungus must be really immortal. Each stage in its development requires an alteration of the external conditions, and the genesis of its reproduction is the beginning of its death.

The second stage of development, the formation of asexual zoospores, can easily be caused by putting a piece of the fungus from the food solution into pure water. Innumerable zoospores come into existence during the next few days, till the mycelium finally dies. The speed of formation depends on the temperature; at 1 degree it is finished in 48 hours, at 20 degrees in eight hours. The essential cause of the process is the decrease of nutritive substances in immediate proximity to the ends of the filaments. To cause the formation of zoospores it suffices, for instance, to put the fungus from a solution containing 0·01 per cent. of peptone into one containing 0·001 per cent. We can effect the continuance of the formation of zoospores for a long time by cultivating the fungus on solid white of egg, and keeping it in water, which must be changed from time to time. The filaments, being well nourished, grow into the but slightly nutritive water and continue to develop zoospores there as long as the substratum affords the necessary nourishment.

The third fundamental stage of development is the formation of sexual organs. If we want to bring this about, the whole of the growing fungus must be subjected to a slow decrease of its nutrition. Sexual reproduction takes place much better when the external conditions at the same time prevent the formation of asexual zoospores. Both stages are, in every case, independent of one another, because they are controlled by different conditions, and it is therefore possible to separate them. We attain our object by cultivating the mycelium in a solution of leucin or hæmoglobin (hæmachrome), mixed with inorganic salts. After the quick growth of the mycelium in the first four or five days, the quantity of nutritive substances is diminished by degrees, and in consequence of this sexual organs appear in great numbers.

We can replace the sexual stage by the asexual one without difficulty, by putting the mycelium with its oogonia into pure water, in which the ends of the filaments bring the zoospores into existence.

Sexual reproduction, itself a very complicated process, requires the formation of male and female organs. De Bary long since observed that with some species of Saprolegnia egg-cells can mature without fertilisation by male cells, and therefore represent a simple case of parthenogenesis.

When the solutions of leucin or hæmoglobin do not contain inorganic salts, the mycelium produces exclusively female organs, of which the unfertilised egg-cells become ripe spores. Formation of male organs—and therewith fertilisation—takes place when salts, especially phosphates, are added. It follows, from these facts, that the various stages of development can be produced or prevented as we may desire, and also that the succession (order) of the stages can be modified at will.

The typical behaviour of the fungus on the dead insect is only one case among many other potentialities, and is easy to understand from its relation to the alterations of environment. The very strong growth of the mycelium after settling on the insect, is the effect of highly nutritious food. The filaments grow into the water, but in so doing they reach into surroundings of poor nutrition, and must thereupon produce zoospores. The nutritive substances contained in the insect are gradually consumed, and the resulting general decrease of nourishment stimulates the fungus into the formation of sexual organs. After the complete exhaustion of nourishment the death of the fungus (with the exception of the resting spores) takes place.

Numerous other lower plants, such as fungi or algæ, show, in principle, the same relations to environment, provided that they can be well cultivated. In all such cases we observe that the alterations of environment essential to the different stages of development are multifarious. But a result of general importance is obtained from experimental researches. The different types of development are produced by quantitative alterations of those factors of nature which are necessary to all life, such as light, temperature, moisture, chemical composition of food, etc. Just as, in the case of the Saprolegnia, the quantitative decrease of nitrogenous substances is essential for the formation of zoospores, so, with the green algæ, the same process takes place on a decrease of the intensity of light, of the concentration of inorganic salts, or of the temperature. In other cases, an increase in regard to each of these factors is required to bring about the same result. In view of our further discussion, it is important to bear in mind that the sexual reproduction of the green algæ also follows this general rule. The controlling factors are found to be a reduction in the supply of nutritive salts (especially the nitrogenous ones) and an increase in the intensity of light, the efficiency of the illumination being responsible for the formation of organic substances, such as carbohydrates. We shall find that these alterations of environment even play a considerable part with the higher plants.

Whilst with the lower plants the deciding influence of environment is beyond doubt, it looks very much as if, with the higher plants (and similarly with animals), development is independent of it. In the first case, the external influences operate on the simple cells in a comparatively direct manner and in a short time. But the higher plants are composed of different members, such as roots, stems, leaves and flowers, which stand in very close relation to one another and affect one another reciprocally. Each alteration of an external factor has an influence upon all the different organs. Those parts of the plant which really produce all new formations, such as the young embryo in the seed or the germinating point of the buds, are always enclosed and protected, so that all changes of environment are transmitted to them along exceedingly complex lines. Further, it must be added that the special internal state of the plant susceptible to any reaction accounted for by environment is the result of conditions of life which existed long ago. These original influences frequently gave the plant a definite tendency, which it continues to follow even though the external conditions vary considerably. The development of flowering plants, carried out for innumerable generations under relatively equal conditions of life, is generally far more clearly defined than in the case of the lower plants.

In spite of all these and other difficulties, we must try to grasp the fundamental problem by means of experiments. Our experience hitherto encourages us to advance, teaching us that the higher plants too are subject, in principle, to the same dependence on environment as algæ and fungi, and, with the object of showing how far it is possible to-day to alter and to control the development of flowering plants, I will now proceed to explain more exactly the experiments which I have carried out during recent years with the plant-genus Sempervivum.

Sempervivum, the well-known house-leek, lives in our countries on walls or rocks, generally in dry places, and prefers high mountains, such as the Alps. The body of the plant consists of a short thick stem, tightly covered with thick sappy leaves, and is called a rosette. The plants can be reproduced either from seeds, or, what is more convenient for our experiments, by a vegetative process.

In the spring the rosette develops short small runners which, after growing some time, in turn develop rosettes at their apex. The young rosette, having taken root in the earth and become separated from the mother-plant, repeats in the next year of its life the same process of reproduction. It is therefore easy to propagate the plant, and all my material for the experiments with the species *Sempervivum Funkii* is derived from a single plant.

According to the external conditions, a rosette can bloom in the third or fourth year of its life. Then, without any formation of daughter-rosettes, the stem, hitherto quite short, begins to lengthen, and, after attaining a length of from 12 to 16 cm., commences to flower at the apex. Below it from three to five branches bearing flowers shoot out. After the ripening of the fruit, which takes place poorly with *Sempervivum Funkii* but freely with other species, the whole plant dies at the end of the summer.

The development of the inflorescence requires a long series of internal alterations of the plant. Quite in the early spring the preliminary processes make the rosettes different to those producing runners with new rosettes. This particular state of the plant, destined to bloom later but still without real rudiments, I shall term *ripe to flower*. Hitherto it has not been possible to cause a rosette to flower in the second year of its life, but we can do so with great certainty in the third year by cultivating the plants during the first year in very rich soil, and for the second year in small pots with very little nourishment and in a relatively dry condition.

The principal question now is whether such a plant as Sempervivum flowers from innate necessity at a fixed time or, like the lower plants, can be altered at will in its development. Our experiments prove that a very essential alteration can be attained by various methods. We cultivate a plant, ripe to flower, during the months of February and March in a special soil-bed. The bed itself is heated by pipes buried in the earth, is very rich in manure and moisture, and is covered with glass. Under these highly favourable conditions of nutrition, it is impossible for the plants to flower in the summer, in spite of the advanced preparations for it. The rosettes will be transformed into purely vegetative ones, which grow very luxuriantly and produce daughter-rosettes later on.

Here we have essentially the same behaviour as with the fungi and algæ, which, as we know, under corresponding conditions continue to grow without reproduction. The experiment can be modified without altering the result, when we cultivate a rosette, ripe to flower, from January to March in a hot-house. An average temperature of 20°, taken in connection with the relatively high degree of moisture, also prevents flowering and produces vegetative growth, less, however, than in the warm soil-bed. In April we are no longer able to alter the development by such experiments, because the internal preparations for flowering are far more advancd. We must apply other methods, consisting of diminishing the production of organic substances by light.

The simplest way is the culture in complete darkness at a constant temperature of 26°. By putting rosettes, ripe to flower, into the thermostat every day from the beginning of April we can determine the moment at which the transformation takes place. Experiments, continued during several years, show that about the middle of April (when no rudiments of flowers exist), the rosettes, originally ripe to flower, become vegetative again.

But this result differs from that already explained in an important point. The rosettes lengthen some centimetres, and, when brought later on into the light, form a terminal rosette in place of the flowers. A stay of two or three weeks in darkness during the month of April suffices to cause this alteration of the plant. It is curious that, in spite of the later good culture during the summer, the altered plant does not flower during the same year.

The same result, but with a stronger growth of the axis, is attained by bringing the rosettes, ripe to flower, into a small house of blue glass which according to spectroscopical examination transmits only the more refrangible rays, from green to violet. In comparison with normal white light, the production of organic substances, such as starch and sugar, is diminished under the influence of blue light, as microchemical and macrochemical tests distinctly show. In consequence of this diminished assimilation of carbon dioxide the rosettes become purely vegetative, but they grow better than in darkness, and by the end of the summer can attain a length of as much as 20 to 30 centimetres.

During the same time, and under otherwise equal conditions, the plants were cultivated in houses of red glass, which admitted only the red rays. In this light the carbon assimilation is greater than in the blue light but less than in the white. Indeed, a certain formation of flowers takes place in the red light, but it is less than the normal one. On the other hand, the lengthening of the stem and of the flowering branches is exceptionally favoured, so that the appearance of the whole plant is sometimes very abnormal.

These experiments prove that the transformation of a plant, ripe to flower, into a vegetative one is possible on the one hand by means of an increase of temperature and manure (that is to say, of inorganic salts), and, on the other hand, by means of a decrease of carbon assimilation. We find, in this respect, full conformity with the behaviour of the lower green plants.

The whole process of formation of the inflorescence consists of numerous successive stages, of which we will distinguish only three essential ones; firstly, the lengthening of the axis; secondly, ramification; and thirdly, the genesis of flowers. What we desire to know is whether these stages can be separated from one another as though they were entirely independent. We have just shown that the lengthening of a plant, ripe to flower, can take place without necessarily bringing on the other stages. As a rule, the lengthening is a life-process which is connected with the flowering only in the ordinary conditions of life, but it is, in itself, wholly independent of it. All rosettes, the vegetative ones included, can lengthen in darkness; however, they do so better in the blue light and best of all in the red light. According to the nutritive state of the rosettes and the intensity of the red light the plants cultivated therein obtain different forms. The very young buds springing from the mother plant preserve their character as runners as long as they grow in red light and never develop into rosettes. After some time in normal light they can produce new rosettes, not only on the apex but also at other places, an occurrence never to be observed in nature.

While on the one hand a lengthening without flowering can be attained, it is possible on the other hand to cause a flowering without lengthening. When rosettes ripe to flower are cultivated at the end of April in very warm soil and in strong light, the flowers are developed at the top of the old rosette, without the axis lengthening.

Under ordinary conditions, the lengthened axis of the inflorescence produces from three to five flowering branches. The number of these branches and of the flowers can vary in a high degree, for example when cultivated in a refrigerator all the branches are suppressed and the inflorescence bears a single flower. With a very vigorously fed plant we observe the production of numerous branches and flowers. In this case the flowering branches proceeded not only from the axils of the highest leaves near the apex but also from those of the middle leaves. The general capacity of all leaf-axils to produce new buds is only realised in experiments in which either the flowering rosettes are continuously fed on inorganic salts or when the top of the inflorescence is cut off. In consequence of this injury, new branches or single flowers are to be found on the whole stem in the axils of all the leaves. The flowers appear also in the axils of the old leaves of the rosette itself, when we extirpate the terminal bud of a rosette, ripe to flower but not yet lengthened, and cultivate it in strong light.

We have found that the terminal flower can be replaced by a leaf rosette under the influence of darkness or blue light. From these facts we can draw the conclusion that at all other places suitable for the genesis of flowers rosettes can be caused quite as well. In all such cases we meet with a certain difficulty due to the peculiar quality of plants known as polarity from the researches of Vöchting. On the stem of a potato plant we observe that the different places produce different organs, as, for instance, on the basal parts, colourless runners and later on potatoes, while from a higher level of the foliage shoots or flowers arise. A similar phenomenon can be observed with the stem of Sempervivum, whose basal parts have a tendency to form rosettes, whilst the apical ones tend to form flowers. In opposition to the ideas of Vöchting, who ascribes the polarity to innate hereditary causes, I assume that the external conditions at the different places of a stem cause internal differences and thereby the formation of different organs.

At the basis near the soil the freer supply of water and nutritive salts favour the formation of rosettes, whereas the apical parts more distant from the soil, in drier air and receiving more light, give birth to the flowers.

These views are verified by the fact that in the experiments rosettes can arise anywhere in place of flowers. A plant, ripe to flower, but made vegetative by blue light, when brought later on into an intense white light forms rosettes in all leaf axils. Similar rosettes, continuously and vigorously fed during the formation of the inflorescence, produce new rosettes at the basal parts in the axils of the old leaf, after we have cut off the inflorescence. But it is also possible to cause the formation of rosettes on the apical branches, and, according to the state of the plant and the variously combined influences, we succeed in obtaining manifold combinations of rosettes and flowers on the same plant.

Under ordinary circumstances the whole plant dies after the flowering and the ripening of fruit. This natural death is not at all a consequence of an innate necessity, but only follows from the reproduction taken in connection with the limited nutrition. All the plants which were compelled by environment to form rosettes in place of flowers continue to live during the winter under very careful treatment. The stem becomes stronger and thicker, the rosettes go on growing, we get peculiar forms (for instance, a very diminutive tree, which also can live until the following year, and perhaps even longer). Such plants come to flower after two or three years and form a new inflorescence on the old stem.

The flowers, the essential parts of the inflorescence, represent organs with a very complicated structure, which is generally so constant for every species that it can be used as the most important factor in the determination and classification of the plants. However, this constant behaviour will be observed only under the ordinary conditions of life. As soon as we apply, at a suitable moment, special combinations of external influences, the flowers are subject to a variability even more intense than that of the vegetative organs themselves.

The production of normal flowers depends, as we know, upon a certain retrenchment of nutritive salts and on an increase of the organic substances; conversely, it is prevented by rich manure and moisture or by a decrease of carbon assimilation. When we produce the conditions so favourable to vegetative growth, either just before or during the first beginnings of flowers, the flowers become altered in every respect and in a high degree.

The best method is to let a well-fed rosette develop into an inflorescence and then to cut off its top at the time of flowering. We further cultivate such plants in soil rich in manure and moisture, and in combination with a high temperature or red or blue light.

In the leaf axils of the reduced inflorescence new flowers take birth under strongly altered conditions and then offer a surprising richness of variation. I have not the time to enter into all the details, but will only deal with some of the more important points. The flower of *Sempervivum Funkii* has a very regular structure, consisting of the same number of sepals, petals, and carpels, and of twice the number of stamens. In the altered flowers the number of all these parts, varying independently of one another, shows strongly marked deviations. Perhaps the various modifications of form in all the members of a flower are of even greater interest. We observe the transformation of sepals into petals, of petals into stamens, of stamens into petals, of stamens into carpels, and of carpels into stamens; in short, we find most of the deviations long known as teratological cases in the garden plants. But with Sempervivum the majority of the deviations had not previously been seen, neither under natural conditions nor under cultivation. They were first brought to light through the influence of external factors.

Among such deviations the formation of double flowers by the transformation of stamens into petals is particularly striking. As far as we know, it does not occur in the Sempervivum of nature, while it is very frequent in the altered flowers.

This rapid sketch of my experiments with Sempervivum, of which the results are verified with other species, will show you, as I hope, what a decisive influence environment has on the development and form of a flowering plant. The forms to be observed in nature correspond to the conditions. Only by quantitative alterations and new combinations of the common factors can we recognise the inexhaustible richness of the potentialities lying in the specific inner structure of the cells.

Our scientific task consists in determining as exactly as possible the part played by all external factors, and (what is far more difficult) in examining the internal alterations, the results of which are the appearance of a definite form. This physiology of development and form is a new branch of modern biology, investigated by botany and zoology at the same time.

During my explanation you will probably have asked yourselves what connection such results have with the question of the transformation of species. The immortal work of Charles Darwin has had a far-reaching influence on all branches of biology, and has also given a fruitful impulse to the physiology of development. But to avoid disappointments and misunderstandings, we must bear in mind that the study of the alterations of plants described by me has nothing to do directly with the question, and is also wholly independent of any theory. We start from the fact, verified times without number, that every species has a constant innate structure, of which the reactions and variations depend on certain factors. All the alterations of the Sempervivum take place only under definite conditions and are replaced by others under different conditions.

A hereditary transmission of these alterations is improbable in the highest degree, and has never been observed. But the question arises whether it is perhaps possible through particular effects of environment to cause internal changes which are transmissible to the descendants. From horticultural experience we know of the appearance of some hereditary modifications, called by De Vries "mutations." We observe, for instance, among the individuals of a red flowering race (genus), one which shows at once and in all its offspring white flowers. We can cause the formation of white flowers by high temperature and feeble light as with Sempervivum. But this alteration is only a simple variation which disappears again under ordinary conditions, while the white flowering plant, created as a mutation, preserves its character everywhere.

How such mutations, so important for horticulture, are produced is wholly unknown. Reflecting that such mutations must be caused as a rule by alteration of the sexual parts, there is every prospect of obtaining seeds from the artificially altered plants. For such researches, however, *Sempervivum Funkii* is not suitable, as it produces few ripe seeds. I used *Sempervivum acuminatum,* another species, which can be altered in the same way as Funkii. Highly altered flowers, with stamens transformed into petals, were carefully self-fertilised. I thus procured a number of seedlings, which were cultivated under ordinary conditions. In the fourth year of their growth the seedlings came to flower. Out of 21 plants, four showed very surprising deviations at the inflorescence and in their flowers. These deviations corresponded to those artificially caused in the mother plant. With two seedlings particularly, all the flowers were altered in the same manner and showed transformations of stamens into petals. A third seedling had flowers, all showing deviations in number and arrangement, and a fourth plant was marked by the formation of small rosettes and of curious intermediate forms.

Beyond all doubt these deviating seedlings sprang from artificially altered flowers, and it seems very probable that these alterations, after having influenced the sexual cells, are transmitted to some descendants. The transmission came to light after four years at the moment of flowering under normal conditions of culture, and without the special methods necessary to the mother-plant having been applied.

Up to the present it is not possible to answer the most important question in the genesis of mutations, namely, whether such alterations can be transmitted to further generations also. These researches are not yet finished, and I mention them only to emphasise the possibilities of experimental study in this direction.

Certainly it seems at present almost too much to hope that Man will be successful in producing new hereditary races artificially, by experimental methods. Nevertheless, the hope of controlling Nature in this respect also is just what continually stimulates the naturalist and gives him strength and courage to strive onward in spite of numerous disappointments and failures.

The Origin and Destiny of Cholesterol in the Animal Organism. Part VII.—*On the Quantity of Cholesterol and Cholesterol Esters in the Blood of Rabbits Fed on Diets containing Varying Amounts of Cholesterol.*

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(Communicated by Dr. A. D. Waller, F.R.S. Received June 17,—Read June 30, 1910.)

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From the study of the inhibitory action of the sera of rabbits fed on diets containing varying amounts of cholesterol on the hæmolysis of blood by saponin we were led to the conclusion, in an earlier paper* of this series, that when cholesterol, free or in the form of esters, is given with the food of rabbits some is absorbed and finds its way into the blood stream as free cholesterol; and also that when phytosterol is used instead of cholesterol it behaves similarly, and some appears in the blood either itself or in the form of cholesterol. The method adopted in these experiments only gave information as to the amount of free cholesterol under various conditions, but not as to the amount of cholesterol esters that might be present. It seemed to us desirable to confirm these results by a chemical method, and also, if possible, to estimate both the free cholesterol and the esters present at the same time in the blood under various conditions.

The usual methods for the chemical estimation of cholesterol are not sufficiently accurate to give reliable information in the case of a fluid such as blood, which contains but a small percentage of cholesterol, and which is only available in relatively small quantities. The discovery of Windaus† in 1909 that cholesterol, but not cholesterol esters, readily combines quantitatively with digitonin to form a highly insoluble compound, digitonin cholesteride, according to the following equation—

$$C_{55}H_{94}O_{28} + C_{27}H_{46}O = C_{82}H_{140}O_{29},$$

afforded a means of solving the problem.

According to Windaus, digitonin cholesteride is insoluble in water, acetone, ether, ethyl acetate and benzene. 100 c.c. ethyl alcohol (95 per cent.), dissolves at 18° only 0·014 gramme, at 78° about 0·16 gramme; 100 c.c. of 50-per-cent. alcohol, at a boiling temperature, dissolves 0·03 gramme. It is readily soluble in pyridin and slightly so in methyl alcohol. On these properties Windaus based an accurate method* for estimating cholesterol, which he applied to the determination of the amount of free and combined cholesterol in kidneys. The mode of procedure we adopted was worked out before the appearance of Windaus' later paper, and differs in detail from that recommended by him. Our object was to devise a plan suitable for the estimation of small quantities of cholesterol in material limited in quantity, and to ascertain, as far as possible, the limits of accuracy attainable under these conditions.

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* 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 230.

† Windaus, "Uber die Entgiftung der Saponine durch Cholesterin," 'Ber. der Deut. Chem. Ges.,' 1909, vol. 42, pt. 1, p. 238.

Method.—The blood was mixed with plaster of Paris and the dry product finely powdered and submitted to prolonged extraction with ether. For the estimation of free cholesterol the ethereal extract was evaporated to dryness, the residue weighed and taken up with 95-per-cent. alcohol. Excess of digitonin in 95-per-cent. alcohol was then added, and the mixture, after standing some time, was evaporated to dryness in a vacuum desiccator. The precipitate was then washed by decantation with ether into a previously weighed filter paper or Gooch crucible, until the ethereal washings gave no residue on evaporation. The excess of digitonin was then washed away by warm water. In most cases the filtration proved tedious, and it was found more satisfactory, on the whole, to use a tared filter paper, care being taken to subject the tare to exactly the same treatment as the filter paper which received the precipitate. The washing with water was continued until there was no residue on evaporation. The precipitate was then dried in an air-oven at 110° C. and weighed, both drying and weighing being carried out in stoppered glass bottles, as the compound is somewhat hygroscopic. In order to estimate the cholesterol present in the form of esters, the ethereal washings containing the fat and cholesterol esters may be saponified with excess of sodium ethylate, the unsaponifiable matter dissolved in alcohol, and precipitated with digitonin as above. It is preferable, however, should the amount of material available permit, to take a separate quantity of material, and after extracting with ether, saponify the residue, and, estimating the total free and combined cholesterol present, obtain the amount of ester cholesterol by difference.

In order to test the efficiency of the method, the following experiments were carried out:—

I. Experiments using small quantities of pure cholesterol, which were dissolved in alcohol and precipitated directly. The results in the following table were obtained, proving that, where one is dealing with pure chemicals at any rate, the method is accurate:—

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* Windaus, 'Zeitschrift für physiol. Chem.,' 1910, vol. 65, p. 110.

Table I.—Pure Cholesterol.

Method of experiment.	Amount of cholesterol (actual).	Amount of compound.	Amount of cholesterol (found).	Actual difference.
Direct precipitation—				
1. Weighings in Gooch crucible ...	0·01	0·0397	0·00960	−0·00040
2. Weighed in tared filter paper ...	0·001	0·0430	0·00104	+0·00004
3. ,, ,, ,, ...	0·01	0·0413	0·01003	+0·00003

II. Experiments to ascertain how far concordant results could be obtained, in the case of a substance containing a small amount of cholesterol, if small quantities of material were used. For this purpose a specimen of beef suet, which had been recrystallised several times by solution in ether and precipitation by alcohol, was taken. It was practically free from cholesterol esters. The results are given in the following table:—

Table II.

Method.	Weight of fat.	Weight of compound.	Weight of cholesterol (found).	Cholesterol in fat.	Average cholesterol in fat.	Difference from average.
Direct precipitation—				per cent.	per cent.	per cent.
Weighings in filter paper	0·3156	0·0015	0·00036	0·115	0·113	+0·002
,, ,, ,,	0·3134	0·0015	0·00036	0·116	0·113	+0·003
Saponification—						
Weighings in filter paper	0·3120	0·0014	0·00034	0·109	0·113	−0·004

III. Experiments in which weighed quantities of cholesterol were added to amounts of fat considerably in excess, relatively to cholesterol, of the weight of the ethereal extracts usually obtained in our experiments on blood. Duplicate analyses were made of the fat alone, and the fat to which measured amounts of cholesterol were added. The results are given in the following table:—

Table III.

Method.	Weight of fat.	Weight of cholesterol added.	Weight of compound.	Weight of cholesterol (found).	Weight of cholesterol in fat (0·113 per cent.).	Weight of cholesterol less cholesterol in fat.	Error.
Direct precipitation—							
Weighings in filter paper	0·3064	0·0491	0·2014	0·0489	0·0003	0·0486	−0·0005
,, ,, ,,	0·3032	0·1060	0·4646	0·1129	0·0003	0·1126	+0·0069
,, ,, ,,	0·3090	0·0242	0·1060	0·0258	0·0003	0·0255	+0·0013
Saponification—							
Weighings in filter paper	0·3097	0·0555	0·2210	0·0537	0·0003	0·0534	−0·0021

IV. In the material one wishes to analyse cholesterol may be present both free and combined, and it was therefore necessary to test the accuracy of the method as applied to the estimation of pure specimens of esters of cholesterol when in the presence of comparatively large quantities of some substance other than cholesterol, which has to be removed by washing or saponification. For these experiments we used known weights of cholesterol oleate and cholesterol acetate in weighed quantities of fat (beef suet) purified by melting and filtering through glass wool, the process being repeated until the fat was clear. Separate analysis of the fat gave a percentage of total cholesterol of 0·07. The results are given in Table IV—

Table IV.

Method.	Weight of fat.	Weight of ester.	Weight of cholesterol in ester.	Weight of compound.	Weight of cholesterol (found).	Weight of cholesterol in fat (0·07 per cent.).	Weight of cholesterol less amount in fat.	Error.
Saponification—								
Weighings in filter paper	0·7630	0·1178 (oleate)	0·0699	0·2772	0·0673	0·0005	0·0668	−0·0031
,, ,, ,,	0·4516	0·1812 (acetate)	0·1634	0·6412	0·1558	0·0003	0·1555	−0·0079

From these results it is clear that, with the quantities of blood used in the experiments described below, the method is sufficiently accurate to give reliable information as to the variation of the percentage of cholesterol and cholesterol esters in blood under various dietetic conditions.

Though the errors of experiment will be relatively greater the smaller the quantity of material used, we believe that dependable results can, with care, be obtained with so small an amount as 20 c.c. of blood.

V. *Experiments on the Cholesterol and Cholesterol Ester Content of the Blood of Rabbits Fed on Diets containing Varying Amounts of Cholesterol.*—The methods of feeding the animals under experiment were similar to those described in our former paper.* Large healthy rabbits were selected, as nearly the same weight as possible, and great care was taken to keep them in good health, and, as far as possible, under the same conditions. The blood of these rabbits was taken and divided into two portions, as nearly equal as possible, which were weighed and mixed with sand and plaster of Paris, as already mentioned. The mixtures, when dry, were extracted in a Soxhlet apparatus with ether, the ether evaporated to dryness, and the residues weighed. The free cholesterol was estimated by direct precipitation with digitonin in the case of the residue from one portion, and the total cholesterol estimated by saponifying the ethereal extract from the other portion and estimating the cholesterol in the residue obtained after saponification. The cholesterol present in the form of ester was then obtained by difference.

In the first set of experiments four rabbits were fed, for comparison, two on extracted bran alone and two on extracted bran plus a measured quantity of cholesterol. These rabbits were kept under the same conditions, and to be comparable, as far as possible, were killed within about ten minutes of each other.

Rabbit 1.—Fed for five days on extracted bran plus measured amounts of cholesterol, $\frac{1}{4}$ gramme first two days, $\frac{1}{2}$ gramme last three days; total amount 2 grammes. Weight of rabbit = 2·7 kilogrammes, constant during whole experiment. Total weight of blood = 62·461 grammes. Rabbit killed 4 hours 35 minutes after administration of cholesterol.

(*a*) By direct precipitation 25·263 grammes of blood gave 0·0562 gramme ether extract, which gave 0·0511 gramme compound = 0·0124 gramme cholesterol, a percentage of 0·0492 free cholesterol in the blood; and

(*b*) By saponification 37·198 grammes of blood gave 0·0822 gramme ether extract, which gave 0·1342 gramme compound = 0·0326 gramme cholesterol, a percentage of 0·0877 total cholesterol in the blood and 0·0385 per cent. of ester cholesterol by difference.

Rabbit 2.—Fed for five days on extracted bran plus cholesterol, $\frac{1}{4}$ gramme for first two days, then $\frac{1}{2}$ gramme for last three days. Total weight of cholesterol = 2 grammes. Killed 4 hours 50 minutes after administration of cholesterol. Weights of rabbit taken periodically during experiment = 2·9, 2·9, 3 kilogrammes. Total weight of blood = 96·011 grammes.

(*a*) By direct precipitation 51·451 grammes of blood yielded 0·1156 gramme ether extract, which gave 0·1218 gramme compound = 0·0296 gramme cholesterol, a percentage of 0·0575 free cholesterol in the blood; and

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* 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 231.

(*b*) By saponification 44·56 grammes of blood yielded 0·1074 gramme ether extract, which gave 0·1642 gramme compound = 0·0399 gramme cholesterol, a percentage of 0·0895 total cholesterol and 0·0320 per cent. ester cholesterol.

Rabbit 3.—Fed for five days on extracted bran. Weights of rabbit during experiment = 3·3, 3·2, and 3·2 kilogrammes. Total weight of blood = 72·3301 grammes.

(*a*) By direct precipitation 31·8071 grammes of blood yielded 0·0838 gramme ether extract, which gave 0·0594 gramme compound = 0·0144 gramme cholesterol, a percentage of 0·0454 free cholesterol; and

(*b*) By saponification 40·523 grammes of blood yielded 0·0702 gramme ether extract, which gave 0·1126 gramme compound = 0·0274 gramme cholesterol, a percentage of 0·0675 total cholesterol and 0·0221 ester cholesterol by difference.

Rabbit 4.—Fed for five days on extracted bran. Weights of rabbit taken periodically during experiment = 2, 1·9, 2 kilogrammes. Total weight of blood = 43·4464 grammes.

By saponification 22·3374 grammes yielded 0·0518 gramme ether extract, which gave 0·0622 gramme compound = 0·0151 gramme cholesterol, a percentage of 0·0677 total cholesterol.

The same conditions were carried out with regard to another pair of rabbits, one being fed on extracted bran, the other on extracted bran and measured quantities of cholesterol.

Rabbit 5.—Fed on extracted bran for eight days. Weights of rabbit taken periodically = 2·3, 2·3, 2·2, 2·15, 2·2, 2·2, 2·2 kilogrammes. Total weight of blood = 65·54 grammes.

(*a*) By direct precipitation 33·82 grammes of blood yielded 0·0738 gramme ether extract, which gave 0·0630 gramme compound = 0·0153 gramme cholesterol, a percentage of 0·0453 free cholesterol in the blood; and

(*b*) By saponification 31·72 grammes of blood yielded 0·0868 gramme ether extract, which gave 0·0722 gramme compound = 0·0175 gramme cholesterol, a percentage of 0·0553 total cholesterol and 0·01 ester cholesterol.

Rabbit 6.—Fed on extracted bran plus cholesterol for eight days. No cholesterol for first two days, ¼ gramme for three days, and ½ gramme for last three days. Total weight of cholesterol = 2¼ grammes. Weights of rabbit = 2·8, 2·7, 2·65, 2·6, 2·6, 2·65, 2·7 kilogrammes. Total weight of blood = 68·21 grammes. Rabbit killed six hours after administration of cholesterol.

(*a*) By direct precipitation 37·09 grammes of blood gave 0·0996 gramme compound = 0·0242 gramme cholesterol, a percentage of 0·0653 gramme of free cholesterol in the blood.

(*b*) By saponification 31·12 grammes of blood gave 0·1079 gramme compound = 0·0262 gramme cholesterol, a percentage of 0·08425 total cholesterol and 0·019 ester cholesterol.

Table V.—Percentage of Cholesterol in Blood.

Rabbit.	Total.	Free.	Ester (by difference).
1. Fed on extracted bran and cholesterol (2 grammes)	0·0877	0·0492	0·0386
2. Fed on extracted bran and cholesterol (2 grammes)	0·0895	0·0575	0·0320
3. Fed on extracted bran alone......................	0·0675	0·0454	0·0221
4. „ „ „	0·0677	—	—
5. „ „ „	0·0553	0·0453	0·0100
6. Fed on extracted bran and cholesterol (2¼ grammes)	0·0843	0·0653	0·0190

We found in our previous experiments on hæmolysis,* that when phytosterol was given with the food the anti-hæmolytic power of the serum was increased, as in the case of cholesterol. We therefore made a comparison of the blood of rabbits fed with ordinary bran, which contains a phytosterol, and the blood of rabbits fed on extracted bran, with regard to the cholesterol and cholesterol ester content.

Rabbit 7.—Fed on ordinary bran for nine days. Weight of animal during experiment = 2·0, 2·0, 2·1, 2·1, 2·1, 2·1, 2·0, 2·1 kilogrammes. Total weight of blood = 61·9405 grammes.

(*a*) By direct precipitation 31·5175 grammes blood gave 0·084 gramme compound = 0·0204 gramme cholesterol, a percentage of 0·0648 free cholesterol in the blood; and

(*b*) By saponification 30·423 grammes of blood gave 0·0962 gramme compound = 0·0234 gramme cholesterol, a percentage of 0·0768 total cholesterol, 0·0121 ester cholesterol.

Rabbit 8.—Fed on ordinary bran for nine days. Weights of animal = 2·6, 2·7, 2·7, 2·7, 2·7, 2·7, 2·7 kilogrammes. Weight of blood = 71·2945 grammes.

(*a*) By direct precipitation 35·3045 grammes of blood gave 0·1023 gramme compound = 0·0249 gramme cholesterol, a percentage in the blood of 0·0741 free cholesterol; and

(*b*) By saponification of ether washings from (*a*) 35·3045 grammes of blood gave 0·0198 gramme compound = 0·0048 gramme cholesterol, a percentage of 0·0136 ester cholesterol, and (by addition) 0·0877 total cholesterol.

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* *Ibid.*, p. 242.

Table VI.—Percentages of Cholesterol in Blood.

Rabbit.	Total.	Free.	Ester.
8. Fed on ordinary bran	0·0768	0·0649	0·0119
9. „ „	0·0877	0·0741	0·0136

Table VII.—Average Percentages of Cholesterol and Cholesterol Esters in the Blood.

Extracted bran.			Ordinary bran.			Cholesterol and extracted bran.		
Total.	Free.	Ester.	Total.	Free.	Ester.	Total.	Free.	Ester.
0·0634	0·0453	0·0165	0·0822	0·0694	0·0128	0·0871	0·0573	0·0298

These results confirm the conclusion arrived at in our former paper, that the phytosterol of vegetable food can be absorbed during digestion and appears in the blood stream, causing an increase in the sterol and sterol ester content, but do not tell us whether the phytosterol gets into the blood as such, or is changed into cholesterol or simply causes an increase in the amount of cholesterol, since both animal and vegetable sterols form insoluble compounds with digitonin.

In order to obtain information on this point 16 rabbits were fed on a mixture of oats, bran, cooked germ of wheat, and some free phytosterol for six days. They were then killed, and the total blood (1074 grammes) extracted in the manner already described. The ether extract was saponified, and the crude cholesterol recrystallised once from a small quantity of 90-per-cent. alcohol. The product thus obtained was slightly coloured and weighed 0·73 gramme. This was dissolved in 7·5 c.c. ether, and the solution mixed with 7·5 c.c. of a solution of 5 grammes of bromine in 100 c.c. of glacial acetic acid. After standing for 1 hour in a freezing mixture, the cholesterol dibromide which had separated out was filtered on the pump and washed with 0·4 c.c. of glacial acetic acid and 0·4 c.c. of 50-per-cent. acetic acid. This cholesterol dibromide weighed 0·75 gramme, and without further purification melted at 115° C. (pure cholesterol dibromide melts at 120° C.). The washings were mixed with the main filtrate and again allowed to stand in a freezing mixture. A small quantity of matter separated, but was not weighed. The filtrate from this precipitate, which should have contained any phytosterol which might have been present, was boiled for two hours with 5 c.c. of glacial acetic acid and ½ gramme of zinc dust to reduce the phytosterol dibromide to phytosterol. The liquid was filtered from the excess of zinc and poured into water, when a small amount of solid matter separated. This was taken up in ether, and the ethereal solution, after shaking with dilute carbonate of soda to get rid of acetic acid, was dried and evaporated. A small quantity of oily crystalline substance was left. This was boiled for some time with acetic anhydride to convert it into the acetate, and then poured into water. The acetate which separated was crystallised from 95-per-cent. alcohol, and the crystals under the microscope appeared to have the form of large thin plates. These were compared with slides of cholesterol acetate and phytosterol acetate, and appeared to resemble the former more closely than the latter. After crystallising three times from alcohol the crystals melted sharply at 101° C. Cholesterol acetate melts at 114° C., and phytosterol acetate at 127° C. The quantity of material was much too small for any attempt at further purification. The minute quantity of material remaining, along with the residue obtained by evaporating mother liquors, was saponified, and the crude product dissolved in pyridine and treated with benzoyl chloride. On pouring into water a small quantity of solid matter separated. This was mixed with some oily matter, and owing to its small amount was difficult to purify. The crystals which separated from alcohol appeared under the microscope to have the form of square plates similar to those of cholesterol benzoate. There was not enough to determine the melting point.

The crude sterol from the blood therefore consisted almost entirely of cholesterol, and contained no detectable quantity of phytosterol. Had the increased sterol content of the blood of bran-fed rabbits compared with the blood of animals fed on extracted bran been due to absorbed phytosterol, we think we could not have failed to obtain some definite evidence of its presence by the above method.

Conclusions.

(1) The digitonin method for the estimation of cholesterol is very accurate, and is capable of yielding reliable results even when only relatively small quantities of material are available.

(2) When cholesterol is given with the food of rabbits, some is absorbed and finds its way into the blood stream. An increase of both free cholesterol and cholesterol esters takes place.

(3) When animals are fed on phytosterol, this substance is in part absorbed,

and there results an increase in the free cholesterol of the blood. Phytosterol does not appear in the blood as such.

We take this opportunity of expressing our thanks to the Government Grant Committee of the Royal Society for assistance in carrying out this work.

On the Comparative Toxicity of Theobromine and Caffeine, as Measured by their Direct Effect upon the Contractility of Isolated Muscle.

By V. H. Veley, F.R.S., and A. D. Waller, M.D., F.R.S.

(Received June 17,—Read June 30, 1910.)

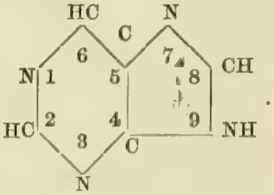
Introductory.

The isolation of caffeine from coffee is generally assigned to Robiquet, Pelletier and Caventou (1821),* though the so-called coffee base, obtained a year previously by Runge† was probably impure caffeine.

The identity of theine, extracted from tea by Oudey (1838),‡ with caffeine was established by the analyses of Jobst (1843).§ The association of caffeine with uric acid was noted originally by Stenhouse,|| who found the latter in a sample of Paraguay tea.

Theobromine was first isolated by Woskresensky¶ from cocoa (1842), and its association with alloxan, on the one hand, and caffeine on the other, was established by subsequent observers.

According to the investigations of Emil Fischer (1894—1898),** uric acid, theobromine, caffeine, and other allied substances, are derived from the parent base purine, to which the following, as one of two alternative formulæ, was eventually assigned :—



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* 'Berzelius Jahresber.,' vol. 4, p. 180, and vol. 7, p. 269.

† 'Phytochemischer Entdeckungen,' p. 144, Berlin, 1820.

‡ 'Mag. Pharm.,' vol. 19, p. 49.

§ 'Annalen,' 1838, vol. 35, p. 63.

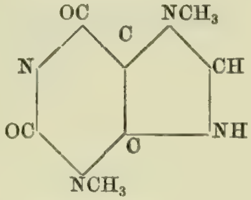
|| *Ibid.*, 1843, vol. 45, p. 371, and vol. 46, p. 227.

¶ *Ibid.*, 1842, vol. 41, p. 125.

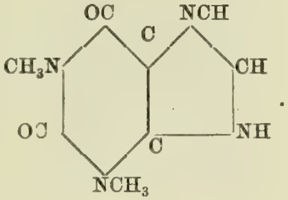
** 'Untersuchungen in der Purin-Gruppe.' Collected volume, Berlin, 1907.

The figures denote the relative positions of the substituting groups proposed originally by Fischer, and now adopted in chemical literature.

Theobromine is 3.7-dimethyl-2.6-dioxypurine, and caffeine or theine is 1.3.7-trimethyl-2.6-dioxypurine:—



Theobromine.



Caffeine.

Hypoxanthine is monoxypurine and guanine its mono-amino derivative, xanthine is dioxypurine and uric acid is trioxypurine.

Unfortunately, the bases guanine and xanthine, as also uric acid, are too insoluble in water (less than 1 part in 1000) for the purpose of our investigation. Among coffee extractives is caffeo-tannic acid, $C_{15}H_{18}O_2$, which occurs as a magnesium or calcium salt in the berries (Rochleder)*; it is a gummy substance readily soluble in water and alcohol.

The substances investigated are theobromine, caffeine, caffeo-tannic acid, and an artificial mixture of caffeine and tannic acid, as also extracts of tea and coffee (with or without caffeine).

Caffeine (*Trimethyldioxypurine*).

All the records obtained were similar in type, the muscle going into contracture, and abolition finally taking place in a longer or shorter time according to the concentration.

In the record given (fig. 1), which is quite characteristic, a *n*/200 solution of caffeine (0·1 per cent.) produced abolition in 11·5 minutes; in other experiments on other days with solutions of the same concentration the times were 10·5 and 11 minutes respectively, and in a fourth experiment on a cooler day, and consequently at a lower temperature (which, however, we omitted to note), the time was 14 minutes. With a solution of double the concentration, namely, *n*/100 (0·2 per cent.), abolition took place in 4 minutes.

In earlier experiments with caffeine hydrochloride and hydrobromide solutions (*n*/200 concentration) contracture took place immediately; this more rapid effect is attributable to the conjoint action of the caffeine and the acid, as these salts under these conditions are nearly completely hydrolysed into their acid and basic constituents.* The above results are summarised below:—

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* 'Annalen,' vol. 59, p. 300.

Caffeine.

Concentration.		Time of abolition in minutes.
Normality.	Per cent.	
$n/100$	0·2	4·0
$n/200$	0·1	11·5
"	"	10·5
"	"	11·0
"	"	14·0

Caffeotannic Acid.

A sample of this substance (Merck), a brown gummy material, as previously described, was made up to $n/100$ concentration (0·32 per cent.) in normal saline, but was found to be practically inactive. A solution of double the strength ($n/50$ or 0·65 per cent.) produced abolition in 21 minutes, the record resembling that of caffeine. By way of comparison a solution was made up of $n/100$ (= 0·2 per cent.) caffeine and 0·5 per cent. of a sample of tannic acid, presumably obtained from oak-bark; the time required for abolition was 20 minutes, namely, five times longer than that for caffeine of the same concentration by itself. The record can be characterised as a retarded caffeine record, suggesting that the free tannic acid added to the caffeine causes some secondary or retarding chemical change.

Theobromine (*Dimethyldioxypurine*).

All the records obtained with this base were similar in type, and resembled those of caffeine; in the record given (fig. 2) for a theobromine solution of $n/200$ (or 0·09 per cent.) concentration, abolition took place in 6·5 minutes. The results obtained are summarised as under:—

Theobromine.

Concentration.		Time of abolition in minutes.
Normality.	Per cent.	
$n/150$	0·12	2·5
$n/200$	0·09	6·5
$n/300$	0·06	8·5
"	0·06	9·0

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* Schmidt, 'Annalen,' 1883, vol. 217, p. 284; Veley, 'Journ. Chem. Soc. Trans.,' 1909, vol. 93, p. 664.

The concentrations are in the ratio 1 : 1·5 : 2 and the times are in the ratio 3 : 2 : 1, with an error of 0·5 minute, which is that of experiment.

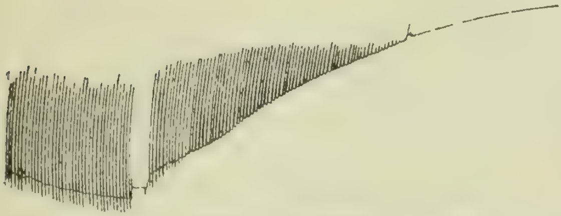

Fig. 1.—Caffeine, $n/200$ (0·1 per cent.).

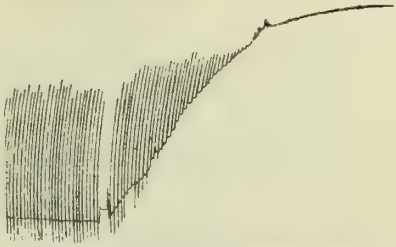

Fig. 2.—Theobromine, $n/200$ (0·09 per cent.).

On comparing theobromine and caffeine solutions of like concentration, namely, $n/200$ (which was actually done simultaneously with a pair of muscles), the times required for abolition are in the ratio 6·5 : 11 or 1 : 1·7; again, on comparing a $n/300$ solution of theobromine with a $n/200$ solution of caffeine the times are in the ratio 9 : 11 or 1 : 1·2; which would be nearly equivalent to a ratio 1 : 1·8 in terms of the same concentration. From these results we conclude that the toxic value of theobromine is to that of caffeine approximately as 1·7 : 1 for equal molecules or 1·8 : 1 for equal weights. It appears that the introduction of the methyl group into theobromine to form caffeine lowers the toxic value, a result which is the converse of that obtained in the case of the paraffinoid alcohols* and unlike those of methyl stovaine and stovaine,† and of pyridine and picoline,‡ in which such introduction of a methyl group produced approximately no alteration of activity.

Coffee and Tea Extracts.

We also made a few observations with ordinary extracts of coffee, with and without caffeine, and of tea; herein we are indebted to the Life Belt Coffee Company, Ltd., for supplying us with samples of "coffee freed from caffeine"; it appears from the analyses given that by the process adopted 90 per cent., or thereabouts, of the caffeine is removed. For the purposes of these experiments we made our "tea" and "coffee" by boiling in physiological saline solution in the proportion of 2·5 and 5 grammes per 100 c.c., filtering off the solid matter and using the extracts. In order to determine the amount of solid dissolved under such conditions 50 c.c. of the 5 per cent. extracts were evaporated to dryness and the residue weighed. After deduction of the weight of the salt in the physiological saline solution, the net weights of the residues (grammes per 100 c.c.) were found to be as under:—

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* Kemp, 'Journ. Physiol.,' 1908, vol. 37, p. 43.

† Veley and Waller, 'Proc. Roy. Soc.,' B, 1910, vol. 82, p. 147.

‡ *Ibid.*, p. 336.

Tea.................................. = 1·98
Coffee.............................. = 1·8
„ free from caffeine......... = 1·48

The following results were obtained, as shown in the records (figs. 3 and 4):—

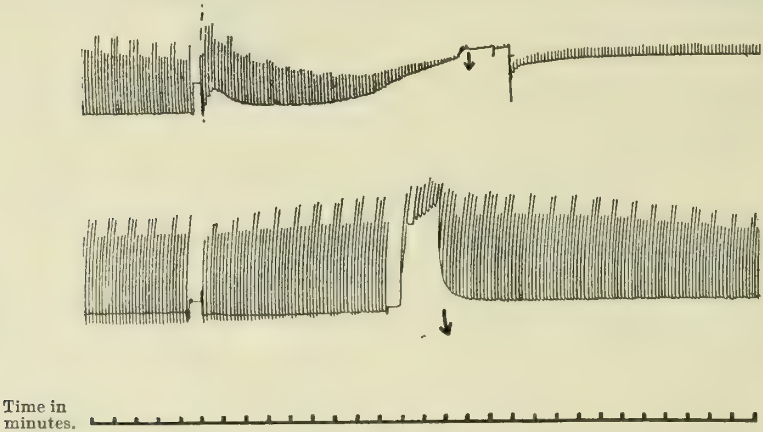

Fig. 3.—Saline Tea. Upper line 2·5 per cent. Lower line 1 per cent., then 5 per cent. (At the points marked by arrows, simple saline was substituted for the saline tea.)

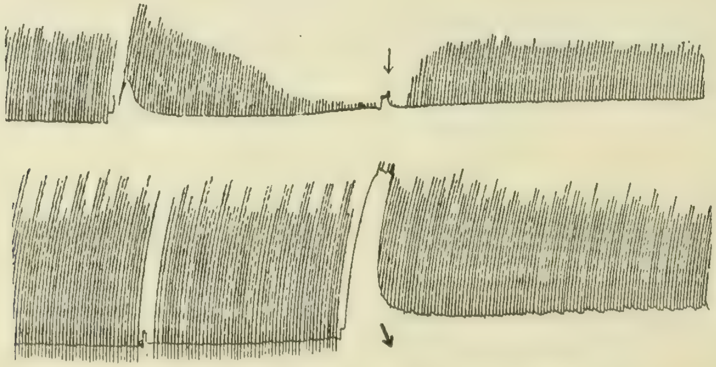

Fig. 4.—Saline Coffee. Upper line 2·5 per cent. Lower line 1 per cent., then 5 per cent. (At the points marked by arrows, simple saline was substituted for the saline coffee.)

Saline Tea Extract.

A 1-per-cent. extract produced no effect; on substituting a 5-per-cent. extract, there was an immediate contracture followed by an equally immediate recovery on replacement of saline solution. After an interval of 19 minutes a 2·5-per-cent. extract was substituted, which caused a slight contracture, abolition taking place in 12·5 minutes, followed by a slight recovery on reintroducing saline solution.

Saline Coffee Extract.

Similarly a 1-per-cent. extract produced no effect; on substituting a 5-per-cent. extract, the effect was almost instantaneous, and the recovery, though incomplete, followed immediately after running in saline solution. After an interval of 19 minutes a 2·5-per-cent. extract was substituted, which caused a contracture with abolition in 12·5 minutes, followed by recovery on running in saline solution.

There do not appear to be any very marked differences between the saline tea and coffee extracts; though possibly the contracture is more marked in the case of the latter, the recovery is less marked in the case of the former. Such a result would be probable, if the toxic effect both of tea and of coffee is mainly due to the caffeine (theine) present, the tannic acid, also a constituent of both, producing only a secondary effect as regards muscle proteins.

But with the extract of the "caffeine-free coffee" (fig. 5) there was no such marked contracture on adding a 5-per-cent. extract, and abolition took place in (nearly) nine minutes; in the case of a 2·5 extract, abolition took place in 21 minutes, followed by recovery on substituting saline solution. The differences between the effects produced by ordinary and "caffeine-free" coffee are most marked, though in the case of the 2·5-per-cent. extracts they would doubtless have been less pronounced if the experiments had been both equally performed with a fresh muscle.

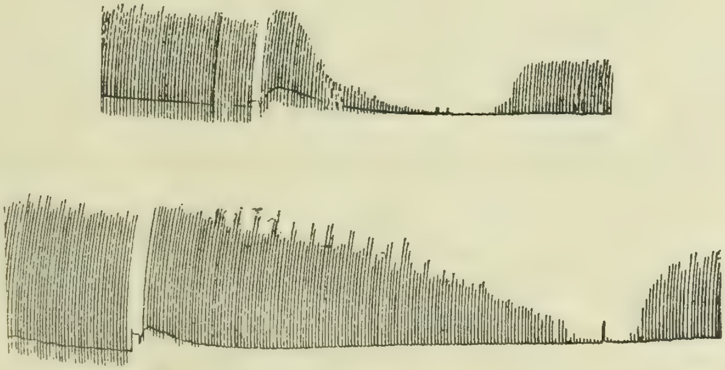

FIG. 5.—Saline Coffee "freed from caffeine." Upper line 5 per cent. Lower line 2·5 per cent.

But, as stated above, the experiments, though striking in their results, were rather of a domestic character than of physical precision. However this may be, the difference between ordinary and "caffeine-free" coffee affords a further proof that the toxic effect is principally due to the presence of the caffeine.

A New Method for the Quantitative Estimation of Hydrocyanic Acid in Vegetable and Animal Tissues.

By Dr. A. D. Waller, F.R.S.

(Received June 17,—Read June 30, 1910.)

(From the Physiological Laboratory of the University of London, South Kensington.)

The evolution of hydrocyanic acid by laurel leaves (*Prunus laurocerasus*) in consequence of congelation, or of their exposure to the action of anæsthetic vapours, was first pointed out by Raphael Dubois.* It has been studied more recently by Guignard,† who has introduced an extremely delicate test for the presence of hydrocyanic acid by sodium picrate paper, and quite recently this test has been applied by F. E. Armstrong‡ for the rapid detection of ferments of the emulsin class. The reaction was first studied by Hlasiwetz§ in 1859, who gave the following equation :—

$$C_6H_3N_3O_7 + 3KCN + 3H_2O = \underbrace{C_8H_4KN_5O_6}_{\text{Potassium isopurpurate.}} + CO_2 + NH_3 + 2KHO.$$

The reaction appeared likely to afford a convenient instance for the simultaneous observation of chemical and electrical changes taking place in living protoplasm under the influence of anæsthetics, and the immediate purpose of this investigation was to determine the parallelism or the want of parallelism between the course of the two changes—chemical and electrical.

Qualitative experiments in which the evolution of hydrocyanic acid from laurel leaves was followed by means of picrate paper, the leaves being enclosed in corked tubes containing the vapour of (1) chloroform ; (2) ether ; (3) alcohol ; and (4) water, gave results in the order of intensity (1), (2), (3), and (4), both as regards evolution of HCN and decline of electrical response. In chloroform vapour the evolution of HCN was greatest, and the abolition of electrical response most rapid. In water vapour there was no evolution of HCN and no diminution of electrical response, so that it appeared at first sight as if the two kinds of effect, increasing in the first case, decreasing in the second, were the associated consequence of the same disturbance of protoplasm.

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* R. Dubois, 'Richet's Dictionaire de Physiologie,' art. "Hydratation."

† Guignard, 'Bulletin des Sciences Pharmacologiques,' 1906, p. 415.

‡ E. F. Armstrong, 'Physiol. Soc. Proc.,' March 19, 1910.

§ Hlasiwetz, Liebig's 'Annalen,' vol. 110, p. 289, 1859.

But on further examination, more especially by quantitative experiments in which the degree of anæsthetic action was graduated and the evolution of hydrocyanic acid estimated, this view proved to be untenable.

In order to make *quantitative* experiments it was necessary to use the anæsthetic reagents in aqueous solution. By preliminary trials it was found possible to do so in weak solution of sodium picrate, which, in the absence of an anæsthetic, was found to have little or no toxic action upon the leaves. A leaf immersed in "picrate fluid"—0·05 per cent. picric acid + 0·5 per cent. sodium carbonate—remains alive for many days, with an undiminished electrical response and without any evidence of HCN evolution, whereas a leaf immersed in the same solution + 0·4 c.c. chloroform per 100 c.c. loses its electrical response and reddens the liquid, in a few hours at ordinary temperatures (16° to 18°), in a few minutes at a temperature of 40°. Similar results are obtained with "picrate fluid" + 5 c.c. ether per 100 c.c., or 20 c.c. ethyl alcohol per 100 c.c.

The varying tints obtained, obviously dependent upon varying amounts of hydrocyanic acid evolved, led me to make experiments with various mixtures containing known small amounts of hydrocyanic acid, in order to see whether a reliable colour scale could be obtained. Equal volumes of picrate fluid and HCN solution (titrated by $AgNO_3$) at concentrations 0·1, 0·01, 0·001, and 0·0001 per cent. gave tints which on appropriate dilution were found to be reasonably concordant, and stable even when exposed to direct sunlight. It was found necessary that the picrate should be taken in great excess in relation to the cyanide, and that in any case, by reason of the slowness of the reaction, comparisons of tint should be taken 24 hours after mixture at ordinary temperatures, or after an hour or two in an incubator at 40°.

The Colour Scale.—The mixture adopted as standard is made from equal volumes of picrate fluid and 0·002-per-cent. HCN left for 24 hours in an incubator at 40°. This mixture contains 10 milligrammes HCN per litre, and has a red colour of an intensity T 10, where T 1 denotes the tint corresponding with 1 milligramme HCN per litre, or 1 millionth gramme per 1 c.c. The colour T 10 is nearly matched by the colour of a 5-per-cent.

solution of potassium bichromate. It is a very "fast" colour—not being appreciably affected by direct sunlight or by boiling.

Thus a tint of T 1 in 1 c.c. liquid represents 0·000001 gramme HCN.
T 1 in 15 c.c. „ 0·000015 „
T 5 in 20 c.c. „ 0·000100 „
T 10 in 100 c.c. „ 0·001000 „

For actual comparison it is preferable to dilute a given liquid, if very highly coloured, so as to bring it below the intensity T 10. For delicate estimations, as *e.g.* of HCN in the blood of an animal poisoned by inhalation of HCN, it is possible to distinguish with certainty tints below the intensity T 1 (*vide infra*, Experiments 11 and 12).

The rate of development of colour in the reaction between picric and hydrocyanic acid is illustrated by the following figure. At 20° the colour of a mixture of equal volumes picrate and HCN (0·002 per cent.) reached one-half its maximal value in 40 minutes, three-quarters its maximal value in 80 minutes. At 40° these times were shortened to 10 and 20 minutes respectively. From which, admitting that the time of intensification to within $1/n$ of maximum depth of tint varies as the logarithm of n, we may calculate times of intensification to within 1/100 of its full depth at different temperatures. Thus the tint of a mixture should reach to within 1/100 of fulness, *i.e.* to 99/100, in 4 hours 46 minutes at 20° and in 1 hour 6 minutes at 40°. Distillates should therefore be left in the incubator for at least an hour before their tints are estimated.

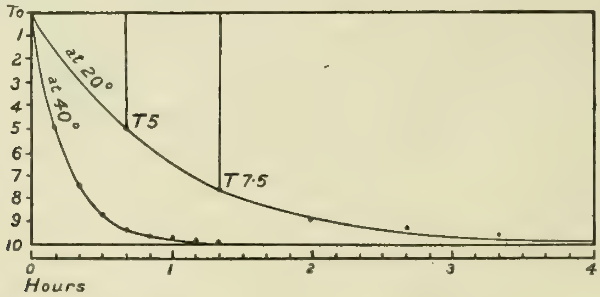

Fig. 1.—Curves showing the rate of development of colour, *i.e.* of the formation of sodium isopurpurate, in a mixture of equal volumes of picrate fluid and of 0·002 per cent. HCN, at 20° and at 40°.

This method, by which it is easy to estimate minute quantities of HCN, counting by thousandths of a milligramme, with an error by manipulation and reading that does not, at present, amount to 10 per cent. of the reading, and that, no doubt, will be reduced by further experience, is very generally applicable. On laurel leaves under the influence of anæsthetics, the daily or hourly evolution of HCN can be followed in a series of tubes from which the contents are decanted at stated intervals (*vide infra*, figs. 2 and 3). On animals (and on man) the tenour in HCN in blood, or in other tissues, can be quantitatively estimated by the colours of distillates from known weights of material into suitable volumes of picrate (*vide infra*, Experiments 1 to 25).

As regards its application to the particular question stated above, as to the parallelism between chemical and electrical phenomena in laurel leaves, it has furnished what, in my judgment, is a clear and unmistakable answer, to the effect that the evolution of hydrocyanic acid—so far from being a sign of life in the sense that the electrical response is a sign of life—is a sign of loss of life, and, at any rate as to its main bulk, a *post-mortem* phenomenon.

On the Time-Relations of Electrical and Chemical Changes taking Place in Anæsthetised Laurel Leaves.

The earliest time after exposure to $CHCl_3$ vapour at which an evolution of HCN can be detected has, in my observation, been 5 minutes. The electrical response to a strong induction shock, *i.e.* the ingoing homodrome or antidrome blaze-current (which, as has been described at length elsewhere,* is a characteristic sign of life), is completely abolished as early as one minute after exposure to chloroform vapour. (Young tender leaves are best adapted to this experiment. Older leaves, by reason of their high electrical resistance, are not suitable.)

We may not infer from the time-difference alone that the evolution of HCN is a *post-mortem* phenomenon, since it may be due to "lag" in the production and diffusion of HCN, and in the reaction of HCN with picric acid.

But a leaf which, after a minute's exposure to $CHCl_3$ vapour, has lost its electrical excitability, and which begins to give signs of HCN 10 minutes later, and goes on giving off HCN for hours and days is assuredly dead. The HCN is then, in the main, a product of *post-mortem* action in a leaf that was killed during the first minute.

The following figures will be sufficient to illustrate the course of this *post-mortem* fermentation, which, like other chemical actions, takes place more rapidly at higher than at lower temperatures:—

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* Waller, 'Lectures on the Signs of Life,' 1903, p. 87 (§ 55), p. 94 (§ 59), p. 109 (§ 65).

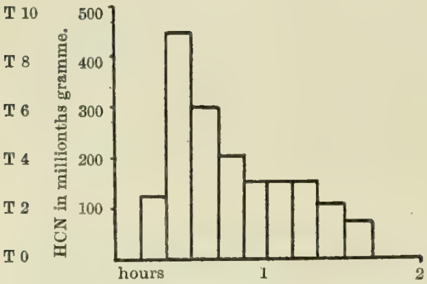


Fig. 2.—Laurel leaf weighing 2·3 grammes, in weak picrate + $CHCl_3$ 4/1000; fluid decanted every ten minutes and compared with a colour scale. Incubator at 40°.

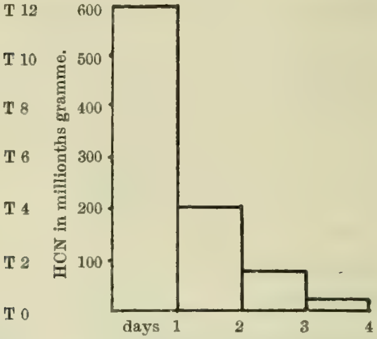


Fig. 3.—Laurel leaf weighing 2 grammes, in 50 c.c. weak picrate + $CHCl_3$ 4/1000; fluid changed each day and compared with a colour scale.

Quantitative Estimation of Hydrocyanic Acid in the Blood and Tissues of Animals, and of Man, after Death by Hydrocyanic Acid Poisoning.

Estimations were made by distilling the blood or minced tissue, mixed with water acidified by tartaric acid, into a solution of sodium picrate. They have been made with the blood and other tissues of animals that had received, by intravenous injection or by injection into the stomach, amounts of hydrocyanic acid, or of potassium or sodium cyanide, much above and rather below the reputed minimum lethal dose; also with the blood and tissues of animals that had died by inhalation of HCN, in whose blood therefore the actual amounts of HCN inhaled had been automatically limited by the arrest of respiration. As will be seen from the following summary of experiments, the method lends itself to the quantitative determination *post mortem* of very minute amounts of hydrocyanic acid. It is applicable up to (if not beyond) 48 hours *post mortem* (Experiment 17). And, as shown in the tabular digest giving millionths ingested per gramme of body weight and millionths found per gramme of tissue, it brings into evidence that the organs in which HCN is found to be most abundant (and for which, presumably, its affinity is relatively great) are the heart and the brain. As shown in Experiment 3, it passes in the blood itself from the plasma to the corpuscles.

The method is simple and expeditious. Each distillation requires about half-an-hour, and, as shown by fig. 1, an hour's digestion at 40° is sufficient to bring out the full (98 to 99 per cent.) depth of tint in the distillate.

Experiment 1.—Cat; weight, 2 kilos.; chloroform anæsthesia. Death by injection into the femoral vein of 10 c.c. of a 1 per cent. solution of HCN. Blood removed from the heart immediately after death and defibrinated.

10 c.c. of blood diluted with water to 75 c.c. *plus* a little tartaric acid, distilled, and the distillate received in three successive portions, each 15 c.c., in 50 c.c. of picrate of soda solution. Next day the three solutions were as follows :—

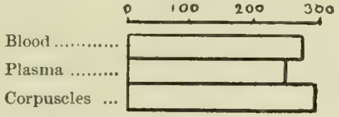
1. The first distillate (65 c.c.) had a red colour = T 8, indicating 0·000520 gramme HCN.
2. „ second „ (65 c.c.) „ „ = T 4, „ 0·000260 „ „
3. „ third „ (65 c.c.) „ „ = T 0·8, „ 0·000052 „ „

Another 10 c.c. of blood was left freely exposed to air for 48 hours and then distilled in two successive portions :—

4. The first portion was of a colour = T 1·5, indicating 0·000090 gramme HCN.
5. „ second „ „ = T 0·75, „ 0·000045 „ „

Experiment 2.—Kitten : 0·6 kilo. ; chloroform ; 10 c.c. of 1 per cent. solution of HCN poured into the stomach. Immediate death. 10 c.c. of defibrinated blood, diluted with water and distilled into 50 c.c. of sodium picrate, gave T 5 (*i.e.* 0·000300 gramme HCN).

Experiment 3.—Cat ; 2 kilos. ; chloroform ; 10 c.c. of 1 per cent. HCN into stomach. Immediate death. Distillation into 25 c.c. picrate.



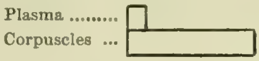
First day distillate—

		Gramme HCN per 10 grammes tissue.
1. From 10 c.c. of defibrinated blood diluted to 60 c.c.	T 9 in 31 c.c.	0·000279.
2. „ 10 c.c. of plasma	T 8 in 29 c.c.	0·000252.
3. „ 5 c.c. of corpuscles	T 5 in 30 c.c.	0·000300.

The difference between blood, plasma, and corpuscles as regards HCN is not very marked.

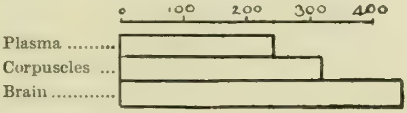
Second day distillate, the blood having been left exposed to air—

4. From 10 c.c. plasma	T 0·75 × 32.	0·000024.
5. „ 5 c.c. corpuscles	T 3·5 × 30.	0·000210.



Experiment 4.—A control experiment. Cat ; 3 kilos. ; killed by chloroform. Distillates into 25 c.c. picrate from 10 c.c. of blood and from 10 grammes of brain. No change of colour in the picrate in either case.

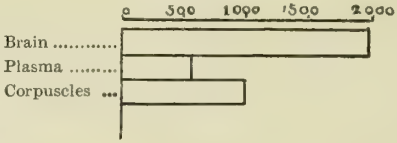
Experiment 5.—Cat ; 3 kilos. ; chloroform ; 10 c.c. of 1 per cent. HCN poured into the stomach ; immediate death ; distillates taken at once.



Distillate 1 from 10 c.c. plasma	gave T 7·5	in 32 c.c.	0·000240.
„ 2 „ 10 c.c. corpuscles	„ T 10	in 32 c.c.	0·000320.
„ 3 „ 10 gr. brain	„ T 15	in 30 c.c.	0·000450.

The remarkable feature here is the large amount of HCN distilled from the brain. Three days later a distillate of the second half of the brain, weighing 10 grammes, gave 0·000045.

Experiment 6.—Cat; 2·4 kilos.; chloroform; 10 c.c. of 1 per cent. HCN injected *per jugulam;* immediate death; brain removed at once; blood defibrinated and centrifuged at once. Materials kept in bottles till next day.



Sixteen hours later—

Distillate 1 from 10 grammes brain gave T 60 in 33 c.c. 0·001980.
" 2 " 10 c.c. plasma " T 18 in 32 c.c. 0·000576.
" 3 " 10 c.c. corpuscles " T 30 in 33 c.c. 0·000990.

So far these are the highest numbers obtained; the exit tube from the still dipped into the picrate solution and there was no appreciable loss of HCN vapour. The colour of the brain distillate was that of port wine; the calculated amount of HCN was nearly 2 milligrammes per 10 grammes (*i.e.* 480 milligrammes per 2·4 kilos. if the poison had been equally distributed over the body of the cat; the actual injection had been 100 milligrammes or 42 milligrammes per kilo. The amount found in the brain was 198 milligrammes per kilo.).

Three days later—

Distillate 4 from 10 c.c. blood gave T 10 in 31 c.c. 0·000310.
" 5 " 10 grammes brain " T 30 in 30 c.c. 0·000900.

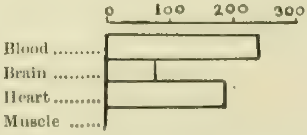
Six days later—

Distillate 6 from 10 grammes brain gave T 10 in 30 c.c. 0·000300.
" 6*a* " 10 " " " T 1 in 30 c.c. 0·000030.

Experiment 7.—Cat; 2·5 kilos.; chloroform; three intravenous injections of 10 c.c. 1 per cent. solution of amygdalin in the course of half-an-hour (= 100 milligrammes = 5·3 milligrammes HCN), immediately followed by 10 c.c. of 0·1 per cent. solution of emulsin. No marked effects. Finally, two injections of 10 c.c. of a freshly made mixture of equal volumes of 1 per cent. amygdalin and 1 per cent. emulsin. Death at the second injection.

The distillate into picrate of 10 c.c. of defibrinated blood gave T 1·2. 0·000072.

Experiment 8.—Cat; 2·4 kilos.; chloroform, 2 per cent.; 10 c.c. of 1 per cent. HCN solution (0·1 gramme) poured into stomach; immediate death. Distillates taken from 10 grammes.



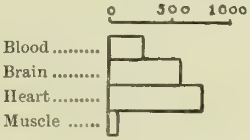
Distillate	1. Blood	T 8 in 30 c.c.	0·000240.
"	2. Hind brain	T 1 in 30 c.c. × 2.	0·000060.
"	3. Fore brain.........	T 1·25 in 30 c.c. × 2.	0·000075.
"	4. Heart muscle ...	T 6 in 30 c.c.	0·000180.
"	5. Gluteal muscle ...	T 0 in 28 c.c.	Nil.

The remarkable point in this experiment was the presence of HCN in cardiac muscle, its absence from skeletal muscle. Subsequent experiments show that this difference is a characteristic feature.

Experiment 9.—Cat ; 2·7 kilos. ; chloroform, 2 per cent. ; 10 c.c. of 2 per 10,000 HCN solution (0·002) injected by the femoral vein lowered the blood pressure ; then 10 c.c. 1 per 1000 (0·01) killed the animal.

Distillate 1.	Blood	T 2	in 30 c.c.	0·000060 gramme HCN.
„ 2.	Brain	T 2	in 29 c.c.	0·000058 „ „
„ 3.	Heart	T 3·5	in 31 c.c.	0·000108 „ „

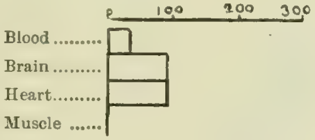
Experiment 10.—Cat ; 3 kilos. ; chloroform, 2 per cent. ; 10 c.c. of 0·5 per cent. HCN solution (0·05 gramme) injected by the femoral vein. Distillates from 10 grammes.



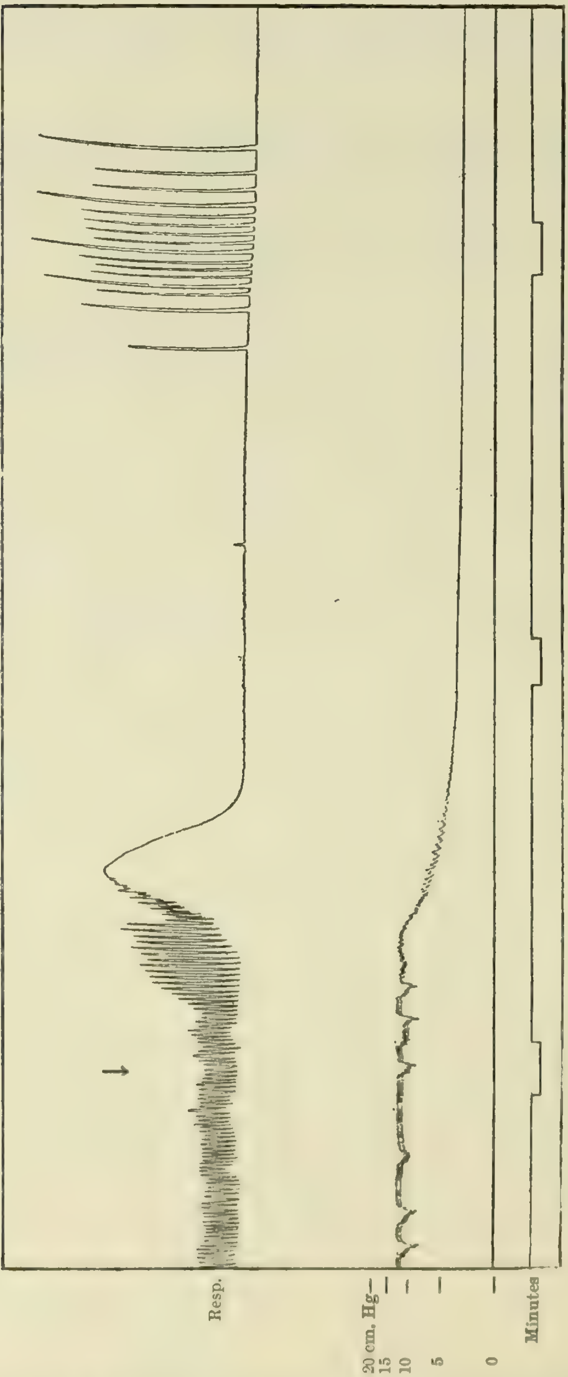
Distillate 1.	Blood......	T 7 × 30 (+T 3 × 30).	0·000300 gramme HCN.
„ 2.	Brain......	T 16 × 31 (+T 3 × 32).	0·000592 „ „
„ 3.	Heart ...	T 25 × 31 c.c.	0·000775 „ „
„ 4.	Muscle ...	T 3·5 × 31	0·000108 „ „

Note.—The heart weighed 12·3 grammes. The brain weighed 23·2 grammes.

Experiment 11.—Cat ; 2·2 kilos. ; chloroform, 2 per cent. Death by injection of 1 c.c. of 0·5 per cent. solution of NaCN (= 5 milligrammes NaCN = 2·6 milligrammes CN). Distillates of 10 grammes into 10 c.c. picrate.



Distillate 1.	Blood	T 1·5 × 20.	0·000030 gramme HCN.
„ 2.	Brain	T 4·5 × 20.	0·000090 „ „
„ 3.	Brain (2nd distillate) ...	T 0 × 20.	Nil.
Distillate 4.	Heart	T 4·5 × 20.	0·000090 „ „
„ 5.	Muscle........................	T 0 × 21.	Nil.



Experiment 11.—Cat. Death by intravenous injection of 0·005 gramme NaCN at the point marked by an arrow.

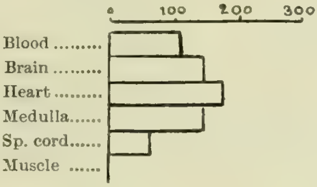
Experiment 12.—Cat ; 2 kilos. ; chloroform, 2 per cent. Death by injection of 1 c.c. of 0·5 per cent. solution of KCN (= 5 milligrammes KCN = 2 milligrammes CN). Distillates of 10 grammes into 10 c.c. picrate.

Distillate	1. Blood	T 2·5 × 21.	0·000052 gramme HCN.
„	2. Brain	T 4·5 × 20 × 10/8.	0·000112 „ „
„	3. Heart	T 2·0 × 20.	0·000040 „ „
„	4. Muscle........................	Nil.	Nil.
„	5. Brain (2nd distillate) ...	Nil.	Nil.

Experiment 13 (June 9, control).—Cat ; 2·5 kilos. ; $CHCl_3$, 2 per cent. for 68 minutes, then at 5 per cent. for 6 minutes. Death. Distillates (20 c.c.) of 10 grammes of blood, brain, and heart into 10 c.c. picrate gave T 0, *i.e.* no HCN.

Experiment 14 (June 9, control).—Cat ; 2·6 kilos. ; ether, 12 per cent. for 23 minutes, then raised to 20 per cent. for 13 minutes. Death by pithing. Distillates as above, of blood, brain, and heart, gave no colour change, *i.e.* no HCN.

Experiment 15 (June 10).—Cat ; 2·8 kilos. ; ether at 20 per cent. for 13 minutes. Respiration stopped, but immediately restored by artificial means. Ether at 12 per cent. Thirty-one minutes later, death by intravenous injection of 5 c.c. of 1 per 1000 HCN (= 5 milligrammes). Distillates as usual.



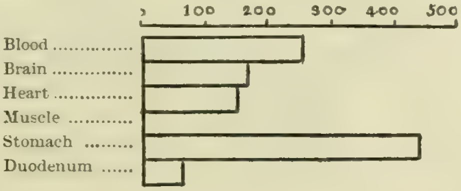
Distillate	1. Blood	T 5 × 22 c.c.	0·000110.
„	2. Brain	T 6·5 × 22 c.c.	0·000143.
„	3. Heart	T 7 × 25 c.c.	0·000175.
„	4. Medulla	T 6 × 23 c.c.	0·000138.
„	5. Spinal cord ...	T 3 × 21 c.c.	0·000063.
„	6. Muscle.........	T 0 × 21 c.c.	Nil.

Experiment 16.—Cat ; 2·6 kilos. Death in one minute by inhalation of 1 per cent. HCN on blotting paper.

1. Blood	T 0·75 × 20 c.c.	0·000015.
2. Brain	T 0·5 × 20 c.c.	0·000010.
3. Heart	T 0·75 × 20 c.c.	0·000015.
4. Muscle	T 0 × 20 c.c.	Nil.

Note.—No estimate is possible of the amount of HCN actually inspired. Judging from the amounts found as compared with those found in the previous experiment, the amount inspired must have been much below 5 milligrammes.

Experiment 17 (June 11).—Cat ; 2·8 kilos. ; ether. Death by 5 c.c. of 1 per cent. HCN poured into stomach. Body left untouched for 50 hours, when the usual distillates were taken, with these results :—



1. Blood	T 9×22 c.c.	2nd dist.	T 3×19	0·000198+0·000057.
2. Brain	T 6×20 c.c.	,,	T 2×22	0·000120+0·000044.
3. Heart	T 6×19 c.c.	,,	T 1·15×22	0·000014+0·000033.
4. Stomach wall	T 15×20 c.c.	,,	T 6×23	0·000300+0·000138.
5. Duodenum wall	T 1·5×21 c.c.	,,	T 1·25×24	0·000031+0·000030.
6. Muscle	T 0×20 c.c.			Nil.

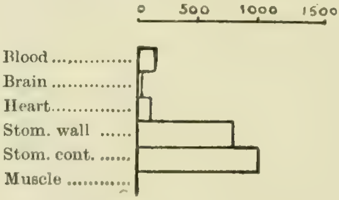
Note.—This experiment shows that after an excessive dose of HCN the poison is discoverable in the walls of the stomach especially, as well as in other organs and in the blood, under the ordinary conditions of a delayed *post-mortem* investigation. It also indicates, by the amounts found in 2nd distillates, that a first distillation may have failed to recover all the HCN present in the blood and tissues.

Experiment 18.—Cat; 2·7 kilos.; ether; tracheotomy. Death in half a minute by inhalation through a Woulfe bottle over a 1 per cent. solution of HCN. Distillates as usual.

1. Blood...............	T 6×21 c.c.	0·000126.
2. Brain...............	T 4×20 c.c.	0·000080.
3. Heart	T 7×22 c.c.	0·000156.
4. Muscle	T 0×21 c.c.	Nil.

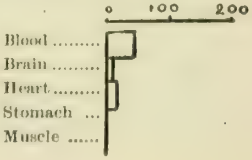
Note.—An approximation to the amount of HCN inhaled was arrived at as follows. The animal stopped breathing in half a minute, having inhaled 600 c.c. of air. A litre of air driven through the Woulfe bottle in one minute into 100 c.c. picrate gave T 120, viz. 0·012000, ∴ the amount inhaled was 0·007200 gramme.

Experiment 19.—Cat; 3·2 kilos.; urethane and chloroform. Death after three successive injections into the stomach of 5 c.c. 1/1000 HCN (in all 15 milligrammes). Body set aside for 24 hours. Distillates as usual.

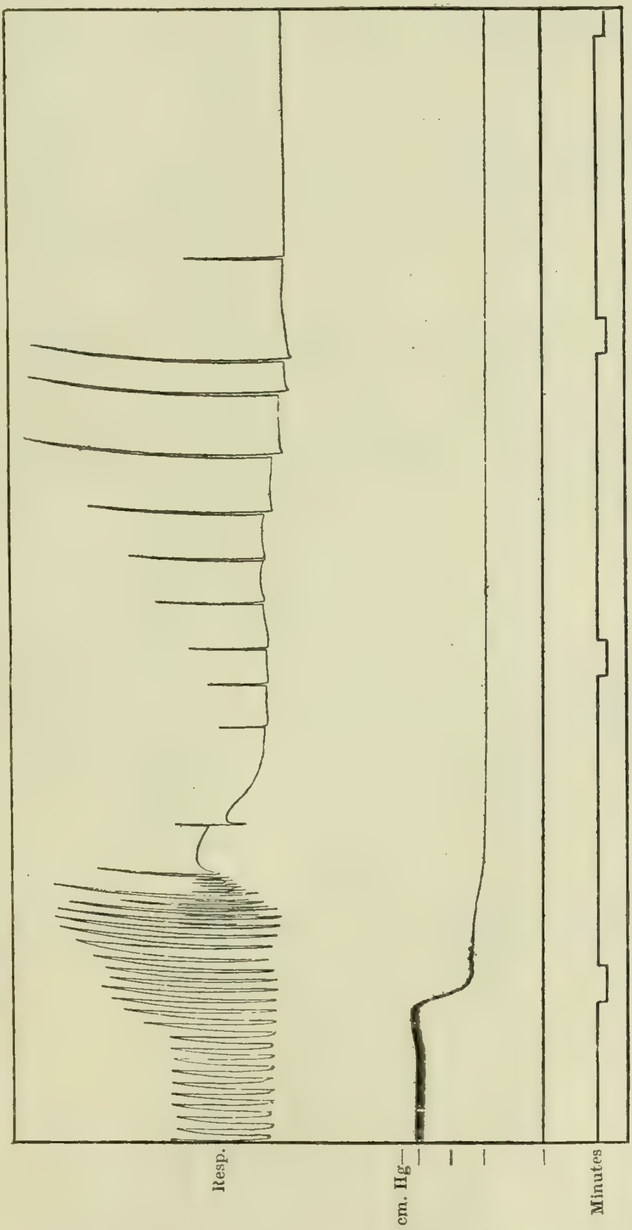


Blood (taken before administration of HCN)...	T 0×20	Nil.
,, (after death)	T 7×22	0·000154.
Brain	T 0·75×23	0·000017.
Heart..	T 5×22	0·000110.
Stomach wall	T 30×21+T 5×22	0·0006300+0·00011.
,, contents (consisting of 8 c.c. liquid)...	T 40×20+T 6×21	0·000800+0·000126.
Muscle	T 0×20	Nil.

Experiment 20 (June 15).—Cat; 2·9 kilos.; ether. Death by subcutaneous injection of 1 c.c. 1 per cent. HCN (= 10 milligrammes).



Distillates of blood............	T 2 × 20	0·000040.
„ brain............	T 0·5 × 20	0·000010.
„ heart............	T 1 × 20	0·000020.
„ stomach	T 0 × 20	Nil.
„ muscle	T 0 × 20	Nil.



Experiment 18.—Cat. Death by inhalation through a Woulfe bottle containing a 1 per cent. solution of HCN. The arrow points to the commencement of inhalation. The sudden fall of blood-pressure occurs 20 seconds later.

Experiments 21 *to* 25 *added to paper August*, 1910.

Experiment 21 (June 17).—Cat ; 3·2 kilos. ; ether. Death in one minute by intravenous injection of 10 milligrammes HCN. Blood drawn off before the injection and after death. Distillates as usual, taken on June 17, 18, and 20.

			grammes HCN.
June 17.—	Blood (before injection)	T 0	0·000000.
	Blood (after death)	T 5 × 25	0·000125.
	Brain	T 8 × 25	0·000200.
	Liver	T 5 × 21	0·000105.
	Spleen (4 grammes)	T 2 × 10/4 × 21	0·000105.
	Gall-bladder (3·3 grammes)	T 0·4 × 3 × 22	0·000026.
	Muscle	T 0·2 × 20	0·000004.
June 18.—	Blood	T 5 × 22	0·000110.
	Brain	T 6 × 21	0·000126.
June 20.—	Blood	T 2 × 20	0·000040.
	Heart	T 5 × 20	0·000100.
	Liver	T 1·5 × 20	0·000030.

Experiment 22 (June 22).—Cat ; 2·5 kilos ; ether. Death in 10 minutes by inhalation over a 1/1000 solution of HCN. Distillates taken same day.

Blood	T 0·75 × 21	0·000016.
Brain	T 0·5 × 23	0·000011.
Heart	T 0·5 × 22	0·000011.
Muscle	T 0·2 × 22	0·000004.
Stomach	T 0·5 × 23	0·000011.
Lungs	T 0·5 × 22	0·000011.

Experiment 23 (June 22).—Cat ; 2·3 kilos. ; ether. Death in 10 minutes by inhalation over 1/1000 solution of HCN. Distillates as usual.

Blood	T 0·75 × 21	0·000016.
Brain	T 2·0 × 22	0·000044.
Heart	T 1·5 × 23	0·000034.
Lung	T 0·75 × 22	0·000016.

In this as well as in the preceding experiment there was more HCN in the heart and in the brain than in the lung, although the poison had been taken in by inhalation.

Experiment 24 (June 23).—By courtesy of Dr. Freyberger. Male, aged 60, found dead in bed on the morning of June 21. Distillates as usual.

Blood	T 2 × 20	0·000040.
Brain	T 1 × 20	0·000020.
Heart	T 2 × 20	0·000040.
Muscle	T 0·5 × 20	0·000010.
Viscera	T 20 × 20	0·000400.

Experiment 25 (June 25).—A control observation, by courtesy of Dr. Freyberger. Female, aged 50. Death under chloroform. Distillates as usual. No sign of HCN in blood, brain, heart, muscle, or viscera.

Expt.	HCN injected, expressed in millionths gramme per gramme body weight.		HCN found, expressed in millionths gramme per gramme tissue.				
			Blood.	Brain.	Heart.	Muscle.	
1.	By vein	50	83	—	—	—	
2.	„ stomach	167	30	—	—	—	
3.	„ „	50	28	—	—	—	plasma 25, next day 2·5; corpuscles 30, next day 21.
4.	Control	0	0	0	—	—	
5.	By stomach	30	—	45	—	—	plasma 24, corpuscles 32.
6.	„ vein	42	—	198	—	—	„ 58, „ 99.
7.	„ „ potentially in amygdalin	4	7	—	—	—	
8.	By stomach	42	24	7	18	0	
9.	„ vein	4·4	6	6	11	—	
10.	„ „	16·7	30	59	77	11	
11.	„ „ as NaCN	1·2	3	9	9	0	
12.	„ „ as KCN	1	5	11	4	0	
13.	Control	0	0	0	0	—	
14.	„	0	0	0	0	—	
15.	By vein	1·8	11·0	14·3	17·5	0	medulla 14, cord 6.
16.	„ inhalation	(?)	1·5	1·0	1·5	0	
17.	„ stomach	18	25·5	16·4	14·7	0	stomach 44, duodenum 6.
18.	„ inhalation	(?) 3·0	12·6	8	15·6	0	
19.	„ stomach	4·7	15·4	1·7	11	0	stomach wall 74, stomach content 92·6.
20.	Subcutaneously	3·4	4	1	2	0	stomach 0.

This table, in which the numbers express millionths of a gramme per gramme (or milligrammes per kilogramme) injected and extracted, shows very clearly that hydrocyanic acid goes to some organs (*i.e.* to the heart and to the brain) rather than to others (*i.e.* the muscles). This is particularly evident where the quantity of poison injected has been very small, *i.e.*, in Experiments 11, 12, and 15.

The Origin of Osmotic Effects. III.—The Function of Hormones in Stimulating Enzymic Change in Relation to Narcosis and the Phenomena of Degenerative and Regenerative Change in Living Structures.

By Henry E. Armstrong, F.R.S., and E. Frankland Armstrong, Ph.D., D.Sc.

(Received April 29—Read June 2, 1910.)

[*International Catalogue of Scientific Literature.*

Authors' title slips :—D, M, Q.

Subject slips :—

D 7155 Q 0224	Osmotic effects in the cell.
Q 0295	Action of anæsthetics in the cell.
D 8010 Q 1205	Hormones as stimulants of enzymic change.
D 8030	Metabolism in the laurel leaf under the influence of hormones.
M 3040	Influence of hormones on changes in the plant.]

It is well known that a variety of substances will stimulate plant growth if used in more or less minute proportions, although when used in excess they are active anæsthetics; the acceleration of the flowering process in plants by means of ether vapour is a familiar case in point; animal as well as vegetable tissues are influenced by such substances. So far as we are aware, no analysis of either process—either of that of accelerated change or of that of retarded change—hitherto attempted has been attended with results that are in any way satisfactory; the tendency has been to correlate all such manifestations vaguely with protoplasmic activity and to attach to them a vital significance. The question we would raise is whether, in the case of animals and plants, protoplasm may not serve as an infinitely delicate indicator of change, whether the effects observed be not often the outcome of changes initially simple in themselves, that commonly escape detection because no proper opportunity is given of observing them.

Our attention has been specially drawn to this subject by two striking communications made to the French Academy of Sciences on July 12, 1909, the one by Guignard, the other by Mirande,* on the effect of chloroform and other anæsthetics in stimulating enzymic activity in leaves; in particular we were impressed by the wonderful delicacy and elegance of the method developed by Guignard of demonstrating the liberation of hydrogen cyanide in the case of leaves containing cyanophoric glucosides by means of paper coloured orange-yellow with an alkaline solution of sodium picrate—if subjected to the action of hydrogen cyanide, such paper is at first coloured a brown-orange, then rose-red and finally a dark brick-red. The test is one of surprising delicacy.

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* 'Compt. Rend.,' 1909, vol. 149, p. 91, L. Guignard, "Influence de l'anesthésie et du gel sur le dédoublement de certains glucosides chez les plantes."

Ibid., p. 140, Marcel Mirande, "Influence exercée par certaines vapeurs sur la cyanogenèse végétale. Procédé rapide pour la recherche des plantes à acide cyanhydrique."

Ibid., p. 829, E. Heckel, "Influence des anesthésiques sur les plantes à coumarine."

If a laurel leaf be enclosed in a test tube together with a slip of the moistened paper, no alteration is observed in the colour of the paper even at the end of several weeks; if a minute drop of chloroform be introduced, the colour changes within a very few minutes.

Mirande, in the note under consideration, calls attention to the method as being applicable to the detection of cyanophoric glucosides in plants generally.

Guignard, by whom Mirande's note was presented to the Academy, discusses the subject from a wider point of view, with reference to observations of a similar character which he has had occasion to make in recent years on the decomposition of the glucoside in cruciferous plants. In particular, he calls attention to the similarity in the effects produced by anæsthetics and by cold.

The stimulative influence exercised by chloroform is specially striking in view of the remarkable efficiency as inhibitants of vital activity of substances so inert as chemical agents as toluene and naphthalene. Having long sought for an explanation of such effects, we have welcomed the opportunity afforded by Guignard's test of ascertaining what class of substances would condition enzymic activity in the leaf.

At the outset, in appeared probable that the leaf surfaces and cellular membranes would act as *differential septa* and that the problem might advantageously be treated from the point of view developed by Adrian J. Brown in his recent striking and suggestive communication to the Society* on the selective properties of the membrane surrounding the barley grain. Not only has this proved to be the case but on account of the escape of hydrogen cyanide whenever penetration takes place, of the ease with which the test is applied and its extreme delicacy, leaves of plants containing cyanophoric glucosides are, in not a few respects, superior to cereal grains as test objects; the latter are perhaps best used in determining the effect various solutes have in regulating the extent to which an inflow of water takes place.

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* "The Selective Permeability of the Covering of the Seeds of *Hordeum vulgare*." By Adrian J. Brown. 'Roy. Soc. Proc.,' B, 1909, vol. 81, p. 81; *cf.* "The Origin of Osmotic Effects. II.—Differential Septa." By Henry E. Armstrong. *Ibid.*, p. 94.

The experiments to be described have been carried out with leaves of the Cherry laurel (*Prunus laurocerasus*). These contain *Prulaurasin*,* which Caldwell and Courtauld have shown to be a mixture in equal proportions of the two isomeric forms of mandelonitrile β-glucoside, $PhCH(CN){\cdot}O{\cdot}C_6H_{11}O_5$.† The glucoside is present not only in the leaves but also in the young stem and the older wood and is always accompanied by an enzyme which can determine its hydrolysis; the bursting buds in which the leaves are just beginning to unfold are particularly rich in glucoside.

The leaves were gathered during March and the earlier part of April; they were picked fresh for each experiment; care was always taken to select uninjured leaves.

In making an experiment, the leaf was placed in a test-tube provided with a sound cork; the "anæsthetic" in excess of the quantity required to saturate the air in the tube or sufficient liquid to cover about one-half to two-thirds of the leaf surface was then added and the tube corked; a slip of slightly moistened picrate paper was introduced together with the cork, which held it in place above the leaf. In some cases, the end of the leaf stalk was sealed by dipping it into molten paraffin wax. The tubes were either set aside at the air temperature or incubated at 37°.

In presence of chloroform, the yellow test paper became appreciably red within half an hour at 37°, within about two hours at room temperatures and within six to eight hours at 0°; the rate of change is therefore about doubled by each 10° rise in temperature.

Effect of Vapours.—In our first series of experiments, we ascertained that the various "anæsthetics" used could be grouped in classes in the following manner, according to the degree of rapidity which they determined the liberation of hydrogen cyanide:—

Quickly Active.—Ammonia, carbon bisulphide, chloroform, toluene, ether, amylic alcohol, ethylic alcohol, amylic acetate, ethylic acetate.

Moderately quickly Active.—Benzene, naphthalene, thymol, acetic acid.

Slowly Active.—Carbon dioxide, benzaldehyde, limonene.

Observations were made with most of these substances at room temperatures as well as at 37°. Similar results were obtained by using in place of laurel leaves flax seedlings (*Linum usitatissimum*) and currant leaves (*Ribes rubrum*.)

Effect of Solutions.—Sound leaves may be from one-half to two-thirds immersed in water at 37° during four or five days or at ordinary temperatures during a much longer period without hydrogen cyanide becoming apparent. Dilute solutions of most metallic salts also produce no apparent effect when leaves are kept in contact with them during three or four days; some stronger solutions and salts of a corrosive nature cause the destruction of the membranes of the leaf and rapidly kill it.

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* Hérissey, 'Compt. Rend.,' 1905, vol. 141, p. 959.

† 'Chem. Soc. Trans.,' 1907, vol. 91, p. 671.

Only a few salts can permeate the differential septa of the leaf and function as cyanogenetics; these condition the escape not only of hydrogen cyanide but also of reducing sugar, which passes out into the solution; apparently the membrane breaks down and ultimately the leaf turns brown.

The majority of the experiments with solutions were made at 37°; the conditions under which the leaf was placed were therefore somewhat drastic, so that even substances which did not at first penetrate the tissues sooner or later determined its death by plasmolysis. Indeed, most salts of the heavy metals gain access after 48 hours. In concentrated solutions, even inactive substances cause the breakdown of the tissues; a 50-per-cent. solution of glycerol and a 10-per-cent. solution of sodium chloride, for example, acted in this manner. The substances referred to above as active as vapours are at least equally active in aqueous solution. Urea passes slowly into the leaf from a weight-normal solution.

The following salts have been found to function cyanogenetically:—

Cadmium Iodide.—The activity of an M/20 solution* is apparent at the end of about 10 hours; an M/15 solution is active within 6 hours.

Sodium and Potassium Fluorides.—In the case of M/20 solutions, the effect is obvious after 24 hours; M/2 solutions are active within about 6 hours. Apparently the potassium salt is the more active. The result is of special interest, inasmuch as fluorides are used as disinfectants.

Mercuric Chloride.—An M/20 solution is weakly active within 24 hours; in M/5 solution the action is far more pronounced.

Iodine dissolved in a 5-per-cent. solution of potassium iodide is slowly active.

Ammonium Salts.—In M/20 solution the activity of the chloride is apparent only after an interval of two days, in the case of the nitrate after 30 hours; both salts are active in M/2 solutions within 20 hours.

Ammonia acts not only as vapour but also in solution (N/4, N/12 and N/20), very rapidly causing the leaf to darken in colour; the picrate paper assumes a deep red colour within two hours. There can be little doubt that ammonia acts as such, not as the hydroxide, as M/10 solutions of sodium hydroxide and carbonate are without effect and an N/2 solution of the former is active only after several hours, presumably because the membrane is gradually destroyed.

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* The solutions were made of weight-molecular or weight-normal strength or fractions thereof, *i.e.* the molecular or equivalent proportion in grammes of the substance was dissolved in 1000 grammes of water.

We are therefore inclined to attribute the apparent activity of the two ammonium salts to ammonia liberated by their hydrolysis, as the solutions gradually become distinctly acid to litmus and to phenolphthalein; it was conceivable that the acidity might have been due to the diffusion of acid from the leaf into the solution but this explanation seems to be precluded by the observation that when the leaf is exposed in a 15-per-cent. solution of alcohol no acid passes into the liquid.

Action of Acids.—The leaf septum is affected by acids more readily than is the barley septum, being destroyed by solutions of sulphuric acid of N/20 strength and upwards, all of which condition the liberation of hydrogen cyanide.

Chlorhydric, phosphoric, tartaric and nitric acids are less destructive and do not enter the normal leaf from N/2 solutions; oxalic acid is active and formic, acetic, propionic and butyric acids pass readily into the leaf from solutions of this strength; lactic acid acts less rapidly than acetic; benzoic and salicylic acids (in saturated solutions) also are quite active. Boric acid passes slowly into the leaf from M/2 and M/5 solutions and after 100 hours from M/10 solutions.

The following substances are inactive in dilute solutions:—

- In 2-per-cent. solutions—
  - Phaseolunatin.
  - Salicin.
  - Arbutin.
  - Amygdalin.
  - Formaldehyde.
  - Vanillin (saturated).
- In 5-per-cent. solutions—
  - Glycine (also in 7 per cent.).
  - Asparagine.
  - Dextrose.
  - Sucrose.
  - Glycerol (10 per cent.).
  - Sodium acetate.
  - ,, borate.
  - ,, chloride.
  - ,, nitrate.
  - Potassium borate.
  - ,, bromide.
- In 5-per-cent. solutions—*continued.*
  - Potassium iodide.
  - ,, phosphate.
  - Ammonium oxalate.
  - ,, sulphate.
  - Copper sulphate.
  - Barium chloride.*
  - Silver nitrate.
  - Magnesium sulphate.
- In N/10 solutions—
  - Sodium carbonate.
  - ,, bicarbonate.
  - ,, hydroxide.
  - Barium ,,
- In M/10 solutions—
  - Cadmium sulphate.
  - ,, chloride.
  - Mercuric nitrate.

It is clear from our observations, taken in connection with those made by Adrian J. Brown, that all substances are effective which can penetrate the cellular membranes and enter into the circulation.

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* Slight action was noticeable on exposing the leaf in a 10-per-cent. solution of this salt during 100 hours.

This conclusion is in harmony with that to be drawn from Vinson's recent observations on "The Stimulation of Premature Ripening by Chemical Means," the account of which [published in the February number of the 'Journal of the American Chemical Society' (vol. 32, p. 208)], reached us only when we had practically completed our experiments. Vinson's work relates to the ripening of seedling dates by exposure to various vapours or in solutions. He has tried over 100 substances. Those which he finds to be effective, with very few exceptions, are such as we should suppose would be active. He concludes that the apparent stimulation of ripening depends solely on the killing of the protoplasm—that "in broad terms, any substance which will penetrate the cuticle and kill or stimulate the protoplasm, thereby releasing the previously insoluble intracellular enzymes without rendering them inactive, will bring about ripening, provided the fruits have reached a certain necessary degree of maturity."

The differential septum of the leaf appears to be even more delicately discriminative than is that of the barley grain.

Since Graham's classical researches on diffusion, it has been customary to distinguish two classes of substances—colloids and crystalloids; it now appears to be desirable to divide the latter broadly into two sub-classes according as they will or will not pass through differential septa such as are met with in the barley grain and the laurel leaf. Starling has proposed to apply the term "Hormones" (from *ὁρμάω*, I excite or arouse) to the chemical messengers, which speed from cell to cell along the blood stream and co-ordinate the activities and growth of different parts of the body;* and he has already ranked carbon dioxide among the hormones. It appears to us desirable to apply the term to all substances which pass through membranes such as we have referred to and excite activity within the cell.

As in the case of the barley grain, the entry of the diffusing substance into the leaf appears to condition the more or less rapid entry of water; the substances which exercise this influence, in both cases, are substances which have but slight attraction for water. In other words, the hormones are "anhydrophilic" substances; hydrophilic substances generally fail to penetrate differential septa which are selective to the degree manifest in cereal grains and the laurel leaf.

It is a most striking fact that substances usually considered to have little, if any, chemical activity should be *the most active* in initiating enzymic change in the leaf, *e.g.* toluene, carbon bisulphide, chloroform and ethereal salts. It can scarcely be supposed that such substances directly determine the liberation of the enzyme from the protoplasm—in other words, that initially action takes place merely because of the breakdown of a zymogen under their immediate influence.

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* The Croonian Lectures, on the chemical correlation of the functions of the body, delivered before the Royal College of Surgeons, June, 1905.—'The Lancet.'

It is generally considered that, in plant tissues, enzyme and glucoside are either contained in different cells or in different vacuoles in the same cell. According to Guignard,* in the cherry laurel the enzyme is stored in the endodermis of the veins; the glucoside is distributed in the parenchyma of the leaf. Even if the enzyme were liberated, it is improbable that it would travel any distance or through a cellular membrane. It is more likely that the glucoside is caused to travel to the enzyme; in such an event, the enzyme might still be active while forming part of the protoplasmic complex, if it were exposed at the surface.

We gather that this is the view held by Guignard, as after calling attention to cases in which drops of liquid appear on the surface of the leaf exposed to the anæsthetic, he proceeds to say:—

"On peut donc dire que le contenu cellulaire subit une véritable deshydratation, puisqu'une partie du liquide passe à l'extérieur de la cellule.

"De même, dans le cas des plantes à glucosides, tels que le myronate de potassium ou l'amygdaline, ces principes sont entraînés avec l'eau qui les tient en solution et arrivent au contact des ferments, localisés dans des cellules spéciales: d'où le dédoublement produisant soit de l'essence de moutarde, soit de l'acide cyanhydrique."

And, in summarising his observations, he remarks:—

"Il y a là, en somme, une méthode générale de recherche de certains composés dont la formation résulte de l'action réciproque d'un ferment et d'un glucoside arrivant au contact l'un de l'autre par l'intermédiare de l'eau. Les observations de M. Mirande sont un exemple très intéressant de son application à la recherche des plantes à acide cyanhydrique."

But it appears to us to be necessary to go somewhat further.

Modern experience would lead us to suppose that, in the living cell, the formation of various hydrolytes is promoted by sympathetic hydrolysts under conditions of concentration which are unfavourable to the occurrence of hydrolysis; from this point of view, the conditions of concentration in the cells should entirely determine the process of change in either the one or the other direction—that of hydrolysis or the reverse.

In the case of the laurel leaf, we have no hesitation in attributing the liberation of hydrogen cyanide by neutral substances which enter as vapours, primarily, to the influence these exercise in causing alterations in concentration of the fluids within the leaf, these alterations being of such nature that the glucoside is thereby brought effectively into contact with the enzyme under conditions which favour hydrolysis. All substances which gain access to the cell must have this effect in some degree.

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* 'Journal de Botanique,' 1890, vol. 4, p. 3.

Initially, such changes need not cause any breakdown of the leaf system and may be merely stimulative, or in some cases, perhaps, inhibitive; they cannot continue long, however, without damage to the protoplasmic mechanism and in isolated tissues must soon pass beyond the stage when recovery is possible—the more so because the effect is cumulative, owing to the influence exercised by the products of change in changing the osmotic state.

From this point of view, it is highly significant that two of the products into which the glucoside prulaurasin is resolved, hydrogen cyanide and benzaldehyde, which pass into the leaf as vapours when used as anæsthetics, both condition the breakdown; obviously, if this were due primarily to some chemical interaction, it would scarcely be initiated by the products of change.

Benzaldehyde, being only slightly volatile, is only slowly active but when a laurel leaf is exposed in a moist atmosphere of hydrogen cyanide, it becomes olive brown within 24 hours.

It is even more striking that the effect is produced by carbon dioxide; when leaves are set aside under precisely similar conditions in corked tubes containing air and carbon dioxide (carefully purified by passage through water and through tightly packed moist cotton-wool scrubbers) respectively, in every case those in the latter gas are the first to turn brown; hydrogen cyanide is given off but the change is manifest only after three or four days, or even longer. An aqueous solution of the gas is somewhat more active.

As freshly picked leaves are found to contain as much as 60 per cent. of water, there is no difficulty in accounting for the presence in leaves of sufficient water to pass from one region to another. The fact that the region in which the enzyme is present is a favoured region, in that the solution flows towards it, is perhaps a consequence of the position occupied by the enzyme close to the exterior surface at which the hormone enters and towards which, therefore, the flux of water is necessarily determined.

It is questionable whether when water enters alone it be active in determining change; in any case, the mechanical action of the entering substance is probably not confined to the water in the cell; the issue thus raised, however, is one which we do not propose to consider until we have completed experiments bearing on the problem.

In the case of substances which enter from solution, water passes into the leaf together with the substance which was dissolved in it. To determine the extent to which water is absorbed, leaves which had been soaked in distilled water during 15 minutes were carefully dried between paper and weighed; they were then one-half to two-thirds immersed in solutions of the substances tested—control leaves being immersed in distilled water under similar conditions. After the required period these leaves were removed, dried as before and weighed.

The results obtained are collected in the following table:—

Treatment.	Increase in weight.
	Per cent.
18 hours in water	4·6
24 „ „	5·0
18 „ in chloroform water	19·6
24 „ „ „	17·0
72 „ in a solution of carbon dioxide made from marble and acid	12·1
72 „ in a solution of carbon dioxide taken from a "sparklet" bottle	11·5
72 „ in 3 per cent. sodium fluoride	11·1
48 „ in 2 „ „ chloride	2·0
72 „ in 2 „ „ „	2·8
72 „ in water saturated with coal gas	4·9

Obviously water enters freely only when active substances are present. The appearance of the leaf indicated when water had entered, the veins being swollen. Hydrogen cyanide was liberated only from leaves immersed in chloroform, carbon dioxide and sodium fluoride.

More concentrated solutions of sodium chloride have the contrary effect and withdraw water from the leaf. Thus a leaf immersed during 24 hours at the ordinary temperature in a 10-per-cent. solution weighed 2·098 grammes before and 2·077 grammes after treatment, corresponding to a loss of 1 per cent. Hydrogen cyanide was evolved but the leaf remained green.

When leaves are stimulated, not only is hydrogen cyanide liberated and more or less water absorbed but the amount of "reducing sugar" in the leaf is also increased, owing to the breakdown of the glucoside and of other hydrolytes such as cane sugar. To estimate this change the leaf was cut into small pieces, quickly weighed and extracted with successive small quantities of boiling water so as to put all enzymes out of action as rapidly as possible. Dextrose was estimated gravimetrically in the aqueous extract.

The amount of reducing sugar in fresh laurel leaves was determined at frequent intervals during the experiments; it varied from 3 to 3·6 per cent. The following figures show the alteration produced by anaesthetics:—

	Reducing sugar.
	Per cent.
Chloroform*	5·13
Another leaf	4·24
Hydrogen cyanide	5·4
Another leaf	5·77
Carbon dioxide	3·8

* The action of chloroform in causing the formation of reducing sugar has also been observed in ivy leaves, which contain neither cyanophoric glucoside nor β-enzyme. Whereas freshly picked leaves contained only 3·04 per cent., after treatment 6·24 per cent. of reducing sugar was found to be present. The breakdown of the leaf is also attended by a marked development of the characteristic odour faintly perceived in leaves in which no action has taken place.

A similar development of aroma, accompanied by browning of the leaf, was observed when leaves of the two varieties of laurel, *Laurus nobilis* and *Cerasus lusitanica*, which contain neither glucoside nor β-enzyme, were acted on by chloroform. The browning may be regarded as an indication that even the oxydases are brought into action.

To study the manner in which substances gain entry, parallel experiments were made in which the upper or lower surface was moistened with solutions which it had been found were able to penetrate into the leaf; such are cadmium iodide, sodium fluoride, acetic acid and mercuric chloride.

In every case, action was most pronounced and took place sooner when the lower surface of the laurel leaf was treated but was also noticeable when the upper surface alone was moistened. Owing to its waxy nature the upper surface cannot well be wetted properly and liquid easily passes to the lower surface; recently we have overcome this difficulty by applying wetted strips of bibulous paper to the leaf surface; from the results obtained in this manner we judge that the upper surface is impermeable.

A macroscopic study of the leaf whilst change is taking place in a solution of carbon dioxide reveals at first the appearance of little dots of dark green on the under surface of the leaf; these areas next become brown; subsequently the brown spreads until it uniformly covers both sides of the leaf; at the same time the veins become swollen and very conspicuous on the under side.

If a leaf be immersed in a dilute solution of methylene blue, it becomes stained locally, a series of small blue dots appearing on the under surface precisely similar to the dark green dots mentioned above. Hydrogen cyanide is not liberated in this case; apparently the dye stuff is laid down entirely at the surfaces of entry.

The explanation we have advanced involves the assumption that in all cases in which growth is promoted or a stimulative effect exercised, even by a substance to which merely "mechanical" activity can be ascribed, the concentration is lowered to a point which is either favourable to a change previously only in the potential stage, or more favourable to one already in operation.

But such agents often exercise an anæsthetic and even a lethal effect if their action be prolonged—how is this to be accounted for? In plants, anæsthesia is shown by the cessation of protoplasmic movements—as in the well-known case of *Elodea canadensis*, for example. Attention has been drawn by Farmer and Waller* to the effect which carbon dioxide, ether and chloroform have in checking the protoplasmic currents in this plant in comparison with that produced by them in depriving muscle of the power of responding to stimulation. In neither case is the inhibitive effect permanent, recovery following the removal of the vapour.

We would venture to attribute the movements in *Elodea* to molecular interactions consequent on the assimilative activity of the protoplasmic complex. In an assimilating vegetable cell, the osmotic tension must be subject to continual change as molecules of carbon dioxide and other simple materials are removed from solution and are raised to various levels of complexity; as such molecules disappear from solution, others diffuse in from outside sources. The more complex molecules that come into being are in part rendered more or less nearly inoperative by incorporation in the protoplasmic complex but some are highly attractive of water, as are also the products of the down grade changes which occur concurrently with the synthetic operations. The consequence of all these changes is that the solvent water in the cells is in the constant state of flux pictured in the expression $(\mathbf{H_2O})_x \rightleftarrows x\mathbf{H_2O}$; the movements of the protoplasm are presumably conditioned by the numerous exchanges which take place between the hydrolated colloid surfaces and the solution. The stoppage of movements by substances which act as anæsthetics may be ascribed to the increase of osmotic tension which these condition in the solution and the consequent balance of the attraction previously existing between the protoplasmic mass (the spongioplasm) and the liquid.

In the case of muscular tissue, far reaching effects must be produced by the translocation of water consequent on the passage of diffusible substances into the various cellular elements; at first this should stimulate change, much as in the case of the laurel leaf, by promoting enzymic interactions; after a time, as the amount of material capable of undergoing such changes became reduced, however, the excitant would lose its influence and osmotic equilibrium would soon be restored. The normal activity of muscle may be regarded as at least largely determined by and consequent upon the movement of water within its substance. One, if not the chief, effect of electrical stimulation on unexhausted muscle presumably is to cause the liberation here and there of products of electrolysis which serve to direct an influx of water into certain regions, giving rise to local expansions which determine the shortening of the fibres; but when the osmotic tension within the cell is raised by these to a certain maximum, an increase of the excitant would tend to determine an outflow rather than an influx of fluid; at this stage electric stimulation would have little effect.

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* 'Roy. Soc. Proc.,' 1898, vol. 63, p. 213.

The argument may even be extended to the explanation of the phenomenon of narcosis in general, including sleep. In saying this, we are aware that physiologists have deprecated the comparison of sleep with the condition induced by anæsthetics. Thus, according to Halliburton,* "the sleep of anæsthesia is a pathological condition due to the action of a poison. The drug reduces the chemico-vital activities of the cells and is, in a sense, dependent on an increasing condition of exhaustion, which may culminate in death. Normal sleep, on the other hand, is not produced by a poison or at any rate we have no evidence of any poison; it is the normal manifestation of one stage in the rhythmical activity of nerve-cells and though it may be preceded by fatigue or exhaustion it is accompanied by repair, the constructive side of metabolic activity."

But such statements raise many questions. It may be asked: What is a poison? How does a poison act? What are chemico-vital activities? These must be answered before it can be asserted that sleep is not produced by a poison. It has been observed that in deeply anæsthetised animals the dendrites of the nerve-cells exhibit thickenings or moniliform swellings; hence it has been inferred—to quote Halliburton (p. 740)—that "in the waking state, the neighbouring nerve units are in contact with each other; transmission of nerve impulses from neuron to neuron is then possible and the result is consciousness; during sleep the dendrites are retracted in an amœboid manner; the neurons are therefore separated and the result is unconsciousness."

Suppose that a poison such as hydrogen cyanide were to penetrate rapidly into the nerve-cells, such swellings might well take place in the main body of the cell and elsewhere, in consequence of the influx of water conditioned by the presence of the "poison" within the cells; in elongated cells this influx would cause a reduction of length along the major axis and might well determine the retraction of the dendrites. Such a messenger (Hormone) would interrupt communication in the nervous system instead of promoting it.

Fatigue diminishes the readiness of the central nervous system to respond to nervous stimuli; presumably, however, fatigue is conditioned not merely by the exhaustion of the supply of changeable material but is also consequent on the production during muscular exertion of diffusible materials (carbonic acid, lactic acid, etc.), the presence of which will necessarily determine the flow of water into the cells in which they are formed—such substances may well be ranked as poisons, as their effect would be to cause the separation of the neurons contemplated above. During sleep such substances would not only be removed but sooner or later synthetic changes would also set in and prevail, so that gradually the osmotic tension would be relieved and the neurons would again be brought into normal relationship.

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* 'Physiology,' 9th edition, 1909, p. 742.

A similar explanation may be applied to the regulation of respiration in animals. As Haldane and Priestly* have shown, even a very slight rise or fall in the partial pressure of the carbon dioxide in alveolar air causes a great increase or diminution, as the case may be, in the alveolar ventilation, the respiratory centre being exquisitely sensitive to any rise in the pressure of the carbon dioxide. All the available evidence, it is said, points to the conclusion that the arterial blood alone furnishes the connection with the respiratory centre and is the carrier of the stimuli. While prepared to agree that carbon dioxide is the normal stimulant, we are of opinion that such substances as lactic acid—indeed, all the products of "fatigue" which can function as hormones—must contribute to the effect.

The physiological effect of alcohol, of drugs such as caffeine, indeed, of most drugs, of flavours and odours as well as that of the presence of more or less digested food in inducing the liberation of the required enzymes in the several regions of the digestive tract, in fact, the effect of hormones generally, is more or less completely accounted for by our hypothesis. Not a few drugs and selective hormones such as adrenaline must exercise, in addition, special effects in virtue of their affinity to certain regions† and it may be that their action is in part inhibitive—similar to that which is exercised by a carbohydrate material "sympathetic" with a given sucroclastic enzyme in retarding the hydrolytic activity of the enzyme. But we prefer to postpone the discussion of these problems until we are able to offer further evidence bearing directly on the subject that experiments we have in contemplation may be expected to furnish.

The activity of substances which function as *hæmoclasts* (hæmolysins) and *bacterioclasts* (bacteriolysins) is probably to be explained without difficulty from our point of view.

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* 'Journal of Physiology,' 1905, vol. 32, p. 225.

† The reason why chloroform and alcohol, for example, affect certain regions preferentially—brain and nerve cells—is probably not far to seek: solvent and solute must always be reciprocally attractive; as the regions specified are rich in fatty matters, of which alcohol and chloroform are special solvents, they are doubtless specifically attractive of such substances, so that these tend to accumulate in them.

One other case we may refer to—that afforded by the fell disease, Diabetes. It is apparently impossible to account for the disposal of the whole of the sugar derived from the digestion of food on the assumption that it all passes as such directly into the circulation; the amount present in normal blood being always very small and subject to but slight variation. The veteran student of this subject, Pavy, has suggested* that as dextrose passes from the digestive tract into the lymphatic system it becomes attached to the lymphocytes; by these it is carried into the blood stream in a hidden form, ready to be liberated whenever and wherever required. The explanation is entirely rational. Assuming it to be correct, the association of dextrose with the lymphocytes must be dependent on a certain high minimum concentration of the lymph; if anything occurred to reduce the concentration below this minimum, the synthetic action would be inhibited and the dextrose would enter as such into the circulation. It is conceivable that the disease is conditioned by the presence of some hormone which determines a reduction in the concentration of the lymphatic fluid. It is at least worth while to study the problem from this point of view.

It is to be supposed that hormones exercise a determining influence in regulating metabolism in plants as well as in animals.†

Obviously, there are many directions in which our hypothesis may be usefully applied. Stated briefly, it is that substances generally which are not attractive to water when introduced into the living cell exercise stimulative effects which are primarily mechanical, as the effects are produced by the interposition of the hormone molecules between the molecules of liquid in the cell; as such interposition, however, at once alters the osmotic state by promoting dissociative chemical change in the water in the direction $(\mathbf{H}_2\mathbf{O})_x \rightarrow x\mathbf{H}_2\mathbf{O}$, the activity of the medium is raised and the equilibrium disturbed, so that an influx of water from other regions sets in. It may be that often the mere dilution thus effected is determinative of change. In some cases contact becomes established between hydrolyte and hydrolyst, by the former passing to the surface at which the hormone is at first effective and which happens to be the seat of the hydrolyst; degenerative changes are thereby set up which tend to increase in intensity, as the products of change in turn exercise a similar stimulative influence and enzymes are gradually set free which can attack the various hydrolytes stored in the cell.‡ Under normal conditions, the products of change pass into circulation and are gradually eliminated, either because they are assimilated elsewhere or because they are excreted. There are undoubtedly other effects to be taken into account but we reserve the discussion of these until we are able to deal with them on an experimental basis.

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* 'The Lancet,' November 21 and 28, December 12, 1908.

† This section is perforce omitted to shorten the communication.

‡ The experiments made by Vernon (*cf.* 'Intracellular Enzymes,' London, John Murray, 1908) are of special interest in this connection. According to this observer, the mammalian heart or kidney may be perfused with the saline solution used by

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The Inorganic Composition of the Blood in Vertebrates and Invertebrates, and its Origin.

By Prof. A. B. Macallum, Ph.D., Sc.D., F.R.S., of the University of Toronto.

(Received June 23,—Read June 30, 1910.)

I.—*Introduction.*

The first suggestion bearing on the origin of the inorganic composition of the blood of animals was that made by Bunge,[*] who, pointing out that we have inherited the notochord and the branchial clefts from marine ancestors, asked why the high percentage of sodium chloride in our tissues should not be an heirloom from life in the sea of that remote past. Eight years later R. Quinton[†] enunciated the view that in the great majority of animal organisms the internal medium, the circulatory fluid, or hæmolymph, is from its inorganic composition but sea water. In support of this he advanced a number of facts bearing on the composition of the internal medium of animals as compared with the composition of sea water, but the parallelism was only in a few instances extended beyond the amounts of sodium chloride in the two media. This view he unfortunately overlaid with a number of speculations, some of which prejudiced its acceptance amongst physiologists and biologists, and in consequence it did not attain the currency to which it was entitled.

In 1903, in discussing the inorganic composition of certain Medusæ the

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physiologists without suffering material change; if however, the solution be saturated with chloroform or ether, the tissue breaks down very rapidly, the proteins passing into solution. The only agent comparable in disintegrating effect with chloroform and ether is ammonia, according to Vernon.

[*] 'Lehrbuch der Physiologischen und Pathologischen Chemie,' Leipzig, 1889, pp. 120 and 121.

[†] 'Comptes Rendus Soc. de Biol.,' 1896, 1897, 1898, 1899. 'Comptes Rendus Acad. Sci.,' vol. 131, pp. 905 and 952. Also "L'Eau de Mer Milieu Organique," Paris, 1904.

author,* unaware of the speculations of Bunge and Quinton, advanced the view that the blood plasma of Vertebrates and Invertebrates with a closed circulatory system is, in its inorganic salts, but a reproduction of the sea water of the remote geological period in which the prototypic representatives of such animal forms first made their appearance. It was pointed out that in many Invertebrates with a vascular system still freely communicating with the exterior, the circulatory fluid is sea water, and this was probably the case also with ancient oceanic forms. The tissues in these latter had through a long period of time become so accommodated to the composition of the sea water of the period that when the circulatory system acquired the closed condition, the composition of the sea water of that period was, with slight modifications, reproduced in the vascular fluid, and thus transmitted to the descendant forms living in different habitats.

As corroborative of this view it was shown that even between the inorganic composition of the blood serum of mammals and that of the ocean of to-day there is a striking resemblance. This is not in concentration, for the salinity of the ocean is about three times that of mammalian blood serum, but in the relative proportions of the sodium, potassium, and calcium, as indicated thus:—

	Na.	K.	Ca.	Mg.
Blood serum	100	6·69	2·58	0·8
Ocean	100	3·66	3·84	11·99

The resemblances are very close except in the magnesium, but this exception and the minor differences were explained as due to the alteration in composition which the ocean has undergone since the Protovertebrate form arose, for not only has there been an increase in the saline concentration of ocean water, but there has obtained a change in the proportions of the basic constituents. This has been brought about by the continual elimination of the potassium and calcium, and the retention of the sodium and magnesium derived from the river discharge from the land areas of the globe.

In a subsequent communication† these observations were amplified, and evidence was advanced to show that the history of the composition of the ocean fully accounted for the difference between the composition of the sea water of to-day and the inorganic composition of mammalian blood plasma.

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* "On the Inorganic Composition of the Medusæ, *Aurelia flavidula* and *Cyanea Arctica*," 'Journ. of Physiol.,' 1903, vol. 29, p. 213.

† "The Palæochemistry of the Ocean in Relation to Animal and Vegetable Protoplasm," 'Canadian Institute, Transactions,' 1904, vol. 7, p. 535.

A difficulty which lay in the way of definitely establishing the oceanic origin of the inorganic composition of the blood plasma was the fact that no analyses of the inorganic salts of the plasma of any Vertebrate below mammals had then been made. Even of the plasma of mammals only few analyses had been on record, and of these only the more recent were from all points of view wholly acceptable. Amongst these came the analyses by Bunge[*] of the sera of the ox, pig, horse, and dog, by Abderhalden[†] of the sera of the ox, sheep, goat, horse, pig, rabbit, dog, and cat. Of the analyses of the serum of man the more important recorded are those of C. Schmidt[‡] and Bunge.[§]

Though there was in all these analyses a marked similarity of proportions in the potassium, calcium and magnesium in relation to the sodium, it was open to doubt whether these proportions would be found to obtain in the blood plasma of birds, reptiles, amphibia, and fishes.

There have been made a number of analyses of the blood in Invertebrates. Those of Griffiths[||] bear on the blood in a number of Crustacea, including the lobster, and in a number of Mollusca, including the Cephalopods, *Sepia officinalis* and *Octopus vulgaris.* In his results there is a remarkable similarity in the composition of the ash in all these forms, and the only noteworthy difference is the comparatively low proportion of lime (CaO) in Cephalopod blood. How far these analyses are representative one cannot say, for his results have not hitherto been checked, but the composition of the blood of the lobster, *Homarus vulgaris,* as given by Griffiths, does not correspond with what I have found in *Homarus americanus,* and it is, therefore, necessary to hold in suspense any opinion as to their general acceptability.

That one must adopt a critical attitude towards the results of analyses of this kind is emphasised by a consideration of those which have been furnished by a number of observations in this respect made on the blood of a single species. Genth,[¶] Gotch and Laws,[**] and McGuigan[††] have analysed the inorganic composition of the blood of the horseshoe crab, *Limulus polyphemus*, and their results are in agreement only in the most general way. While the discrepancies are in some cases at least extraordinarily great, Genth's results even as regards the composition of the blood of forms from two different points on Chesapeake Bay are quite unlike, and the question is raised whether the variations are due to imperfect methods of analysis or to actual differences in composition. That his methods did not give exact results may be gathered from the fact that the percentages of potassium in the samples of blood from the two different sources were widely different, the element appearing to be twice as abundant relatively in one case as in the other. This is to a certain extent intelligible, for the methods of estimating potassium employed 60 years ago were much less exact than they are now, but even a defective method ought under like conditions to give uniform results.

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[*] "Zur quantitativen Analyse des Blutes," 'Zeit. für Biologie,' 1886, vol. 12, p. 191.

[†] "Zur quantitativen Analyse des Blutes," 'Zeit. für Physiol. Chemie,' 1897, vol. 23, p. 521. Also "Zur quantitativen vergleichenden Analyse des Blutes," 'Zeit. für Physiol. Chemie,' 1898, vol. 25, p. 65.

[‡] "Charakteristik der Epidemische Cholera," Leipzig und Mitau, 1850.

[§] *Op. cit.*, pp. 221 and 222.

[||] 'Roy. Soc. Edinburgh Proc.,' 1890—91, vol. 18, p. 288.

[¶] 'Annalen der Chemie,' 1852, vol. 81, p. 68.

[**] 'British Association,' 1884, p. 774.

[††] 'Science,' 1907, vol. 25, p. 68.

The differences also between the results of McGuigan, on the one hand, and those of Gotch and Laws on the other, would, if accepted, make it impossible to regard the blood in *Limulus* as uniform either relatively or absolutely in its inorganic composition. That would entail the further conclusion that in *Limulus*, after a life in the sea almost co-extensive with geological history, there is a tendency to wide variation in the inorganic composition of the blood, irrespective of the changes in the concentration of the salinity of the sea water of its habitat. If that conclusion were correct it would indeed be difficult to understand how fixed relations in the inorganic composition of body fluids ever could arise, and the primal causation of such fixed relations in, for instance, mammals would be an enigma.

So far, therefore, the results of analyses hitherto made on the blood of Invertebrates leave the question of the origin of the fixed proportions of the inorganic elements in the blood of the higher Vertebrates still in doubt.

In order, therefore, to dispose of the question of the origin of these fixed proportions it was necessary to analyse the blood of representatives of the lower classes of the Vertebrates and of Invertebrate types with a closed circulation. This involved the collecting of quantities of blood plasma from a number of forms, many of which are not accessible ordinarily and, in consequence, the determination of the problem has been delayed far beyond the limit originally set in the writer's plans.

Within the last two years he succeeded in obtaining a quantity of material which has enabled him to undertake a partial investigation of the question. Through the kindness of the Director of the Woods Holl Biological Station he received a large quantity of the blood of the horseshoe crab, *Limulus polyphemus*. During his visit to the Canadian Marine Biological Station at

St. Andrews, New Brunswick, in August of last year, he obtained a quantity of the blood serum of the cod, *Gadus callarias*, also about 200 c.c. of blood serum from the pollock, *Pollachius virens*, and about 500 c.c. of blood plasma from three large specimens of lobster, *Homarus americanus*. Later, through the kindness of the Acting Director of the Station, Prof. Penhallow, considerable quantities of the blood and blood serum of the dogfish, *Acanthias vulgaris*, were collected and placed at the author's disposal.

These have been analysed to determine their inorganic composition, and the results, while limited, and therefore inadequate to determine finally the solution of the question, are contributory to that end and are of sufficient interest to justify their publication.

II.—*Methods of Analysis.*

The methods of analysis followed were on the whole those employed by Bunge in the analysis of the inorganic composition of milk, and subsequently adopted by Abderhalden in his investigations on the composition of the blood in a number of mammals. Modifications were introduced into these methods as the description of them indicates.

For the determination of the potassium and the sodium a weighed quantity, varying in volume from 30 to 80 c.c., of the blood or the serum was evaporated to dryness in a large platinum dish and then, after being heated for five hours at 115° C. to determine the residue, the latter was carefully carbonised at a low red heat till all the volatile organic matter was destroyed. The carbonised residue was then extracted several times with hot water, and the residue, after being dried and incinerated, was dissolved in dilute hydrochloric acid. This latter solution was added to the volume of the extraction fluids, and to the resulting mixture saturated baryta water was added to precipitate the sulphuric and phosphoric acids; the mixture was filtered, the filtrate reduced in volume by evaporation on a water bath, and then treated with crystals of ammonium carbonate to precipitate the calcium, magnesium, and the excess of the barium from the solution. The filtrate from this was evaporated to dryness in a large platinum dish, the residue fused with anhydrous oxalic acid, then dissolved in water and the solution filtered. The filtrate, containing the carbonates of sodium and potassium, was now evaporated to dryness in a small platinum capsule, the residue heated to dull redness and then dissolved in 5–8 c.c. of water. If the solution was not clear it was filtered through a filter paper of smallest possible superficial area, the solution again evaporated to dryness in the capsule and the residue heated to dull redness. If, now, on dissolving this residue in 5–8 c.c. of water, the solution was not clear, it was filtered, the filtrate evaporated, and the residue again heated to dull redness, then dissolved in not more than 5 c.c. of water and the solution filtered. This solution, which contained only the carbonates of sodium and potassium, was treated with hydrochloric acid to convert them into the chlorides; the solution was now evaporated to dryness in a weighed platinum capsule, and the weight of the chlorides of sodium and potassium determined.

To determine the potassium the chlorides were dissolved in water; to the solution 3-5 c.c. of a 10-per-cent. solution of platinum chloride were added, and after the addition also of 3 c.c. of concentrated hydrochloric acid, the mixture reduced almost to dryness in a porcelain evaporating dish on a water bath. The residue was covered with 40 c.c. of absolute alcohol, the mixture stirred for a few moments, and then allowed to stand under a bell jar for an hour, at the end of which time 20 c.c. of ether were added, and a further extraction of an hour was allowed. After decanting the alcohol-ether mixture, a fresh mixture, consisting of 40 c.c. of alcohol and 20 c.c. of ether, was added and allowed to extract for an hour. On decantation of this a third supply was added, and the extraction continued for another hour. The supernatant fluid, which was quite colourless, was also removed by decantation; the residue was then dried, and the platinum salt in it was reduced to metallic platinum by heating it to 250° C. in a current of dry hydrogen. On cooling, the salt was dissolved in water, then evaporated to dryness, and once more subjected to reduction in dry hydrogen gas. This latter procedure was adopted in order to ensure complete reduction of all the platinum salt present.

The weight of the platinum thus found, multiplied by the factor 0·40195, gave the amount of potassium. This factor corresponds approximately to the theoretical value of K in the formula K_2PtCl_6.

The determination of the iron, copper (in Crustacean blood), calcium, magnesium, and phosphoric acid was, except in cases to be mentioned subsequently, made in the following manner:—To the weighed quantity of the blood or serum held in a platinum dish about 2 grammes of pure sodic carbonate were added; the mixture, after being carefully stirred, was evaporated to dryness, the residue carbonised, then extracted several times with hot water, acidulated with hydrochloric acid, the remainder of the residue completely incinerated, the ash extracted with hot dilute hydrochloric acid, the fluid filtered, and the filtrate added to the volume of the united filtrates previously obtained. What remained undissolved was ferric oxide (Fe_2O_3), which was weighed. Into the acid solution, when it contained copper, as was the case when the preparation was derived from Crustacean blood serum, sulphuretted hydrogen gas was passed, the sulphide of copper formed was separated by filtration, and the copper determined as Cu_2S or CuO. The filtrate from this, after being boiled for some minutes to drive off the dissolved sulphuretted hydrogen, was nearly neutralised, acetic acid and crystals of ammonium oxalate were added, and, after standing for some hours, the calcium oxalate precipitate was removed and weighed either as CaO or $CaSO_4$. From the filtrate the magnesium was precipitated as magnesium phosphate by the addition of ammonium phosphate and ammonia.

In the case of the solutions made as described from the ash of Vertebrate blood or serum, ammonium acetate was added, and the mixture allowed to stand for 24 hours in order to precipitate some of the iron and all the phosphoric acid as ferric phosphate, which was removed by filtration. If there still remained iron in solution (as in the case of Vertebrate blood), the fluid was rendered alkaline with ammonia, the resulting precipitate of oxide of iron, lime, and magnesia was removed by filtration and, while still moist on the filter, extracted several times with a hot solution of ammonium chloride, which dissolved out the lime and magnesia and left the ferric oxide. The extract containing the dissolved lime and magnesia was added to the filtrate previously obtained, the whole reduced in volume by evaporation, the calcium precipitated as oxalate and from the filtrate the magnesium as phosphate in the usual way.

Recognising that the precipitate of calcium oxalate obtained in each case might, in spite of the method of separation used, contain also some magnesium oxalate, it was incinerated, the ash dissolved in acetic acid, and ammonium oxalate added to the solution to precipitate the calcium, which was weighed as lime or as sulphate. The filtrate from the precipitate was then appropriately treated, to separate and estimate the traces of magnesium present.

For the determination of the chlorine, 1–2 grammes of sodium carbonate were added to a weighed quantity of the serum or blood contained in a platinum dish, the mixture, after being carefully stirred, was evaporated to dryness, and the residue thoroughly carbonised at a dull red heat. The mass was extracted with hot water several times, and then completely incinerated at a low heat. The ash was dissolved in cold dilute nitric acid, the solution filtered, and the filtrate added to the volume of the extracts previously obtained. The chlorine in this was determined as chloride of silver in the usual way.

The sulphuric acid was determined only in the blood of the lobster and of the horseshoe crab (*Limulus*). A weighed quantity of the blood was slightly acidified with acetic acid and heated, in order to coagulate the proteids, which were then removed by filtration. To the filtrate, which was clear, or had only a faint opalescence, some hydrochloric acid and a solution of barium chloride were added, and, after the fluid was heated almost to boiling, it was allowed to stand for 24 hours, when the precipitate of barium sulphate was removed by filtration and its weight determined.

The determination of that part of the depression of the freezing point (Δ) of a specimen of plasma or serum, due to the salts in it, was carried out in the following way: The weighed quantity of the blood (lobster and horseshoe crab) or serum (cod, pollock, and dog-fish) was evaporated to dryness, first on the water-bath, then in an oven at 115° C. for six hours. The residue was now carbonised at a low heat, and the carbonised residue then extracted several times with hot water containing hydrochloric acid, the remaining material completely incinerated at dull red heat, the ash dissolved in dilute hydrochloric acid, and the solution added to the volume of the extracts previously obtained. This fluid was now evaporated on a water-bath to complete dryness, and the residue then heated carefully, in order to convert any ferric chloride present into ferric hydrate. A few drops of dilute hydrochloric acid were added to dissolve all the magnesium salts present, then the preparation was carefully evaporated and heated to expel all traces of free acid. It was now dissolved in sufficient water to make the total weight that of the plasma or serum taken, and the Δ of this solution was then determined. In the majority of cases, two such solutions were made from two weighed portions of the same plasma or serum.

The values of the Δ so obtained cannot be regarded as free from objection. The solutes in such fluids are not under the same conditions as in the plasma or serum. In the latter, their dissociation is diminished by the colloids present. This, however, is compensated for in such solutions to a certain extent, since, owing to the absence of colloids, there is a slightly greater degree of dilution of solutes, and though there must, therefore, be a very slight increase in dissociation, the depression of the freezing point must be lessened. On the whole, consequently, while the values of the Δ ascertained may not be absolutely accurate, they still are data which can be compared with the values for the blood and serum.

Such solutions were further employed when the quantity of the plasma or serum was limited, as was the case with those fluids from the dog-fish and the cod. In this case the material had to be so used as to permit of the determination of as many constituents as possible. The iron in such solutions was precipitated by the addition of strong acetic acid and some ammonium phosphate, and from the filtrate ammonium oxalate precipitated the lime as oxalate. The magnesium in the filtrate from this was precipitated on the addition of more ammonium phosphate and of strong ammonia, and the filtered fluid evaporated to dryness and heated to expel the acetic acid and ammonium acetate. To the residue, dissolved in a small quantity of dilute hydrochloric acid, saturated baryta water was added to remove the sulphuric and phosphoric acids, the excess of the baryta was precipitated with ammonium carbonate, and the filtrate from this was treated in the way described above, to determine the sodium and potassium.

III.—*The Results.*

A. *The Blood Serum of Limulus.*—The blood of *Limulus* was coagulated, and contained a large quantity of fibrin, which was removed by straining through fine muslin cloth. The serum was deep azure-blue in colour, and had a specific gravity of 1·03847. Its Δ was −2·04° C. The Δ of the salts in the blood was −1·875° C. The total salts amounted to 2·982 per cent. The percentages of solids in four different estimations were 7·960, 7·822, 7·942, and 8·056. The different results in the four estimations were due to differences in the time during which the residue was dried, first in a steam-heated oven, and then at 110° C. The average per cent. of these determinations was 7·945. The analyses of the ash gave the following:—

	I.	II.	III.	Mean.
Na	0·8944	0·8864	0·8848	0·8885
K	0·04951	0·0500	0·05017	0·04989
Ca	0·03614	0·03621	0·03604	0·03613
Mg	0·0995	0·0996	—	0·09955
Cl...................	1·6526	1·669	—	1·6608
SO_3	0·11718	0·11976	—	0·11847
Cu	0·00742	0·00773	0·00806	0·00773
				2·86107

B. *The Blood Serum of the Lobster.*—The blood of the lobster was rich in fibrin, and was of a light blue colour. The specific gravity of the serum was 1·0337. The solids in two estimations were 8·354 and 8·351 per cent., and the mean 8·3525 per cent. The total salts in two estimations were 2·855 and 2·849 per cent., the mean 2·852 per cent. The Δ due to the salts ascertained from these two determinations were −1·73° and −1·735°. The Δ of the serum in two different determinations was −1·78° C. The sea water from the bottom of the bay (at St. Andrews, New Brunswick), where the lobsters were caught, gave a Δ of −1·76°, that of the surface at the same point −1·635°. The analyses of the ash gave:

	I.	II.	III.	Mean.
Na	0·90294	0·90052	0·9065	0·90335
K	0·03491	0·032806	0·03361	0·03377
Ca	0·04394	0·04379	—	0·04387
Mg	0·01636	0·01468	0·01579	0·01561
Cl	1·5501	1·5439	—	1·547
SO_3	0·06107	0·0597	—	0·06038

C. *The Serum of the Dogfish.*—Two determinations of the Δ of the serum gave −2·035°, and two of that of the salts of the serum gave −1·075° and −1·0725°, the mean of the two being −1·0737°. The total salts of the serum in these two cases amounted to 1·775 per cent. and 1·7729 per cent., and the mean was 1·7739 per cent. The total solids, as ascertained in one determination, were 5·956 per cent. The analyses gave:

	I.	II.	Mean.
Na	0·59216	0·59158	0·59187
K	0·02742	0·02717	0·02729
Ca	0·016105	0·016011	0·01606
Mg	0·01402	0·01519	0·0146
Cl	0·9802	0·9837	0·9819

The blood (corpuscles and plasma) of the dogfish gave on analysis the following:—

	I.	II.	Mean.
Na	0·4959	0·4931	0·4945
K	0·0844	0·08255	0·08348
Cl	0·9391	—	—

D. *The Serum of the Cod.*—The solids of the serum of the cod gave in three different determinations 7·165, 7·1006, and 7·170 per cent., the mean of which is 7·1452 per cent. In one determination the total salts were 1·2823 per cent., and this gave a Δ of −0·71° C., while that of the blood of two very large cod, ascertained within two and a-half hours after their capture, was −0·765° C. The chlorides of sodium and potassium present amounted in three determinations to 1·1326, 1·1349, and 1·1293 per cent., the mean of which was 1·1322 per cent. The results of the analyses of the ash were:

	I.	II.	III.	Mean.
Na	0·4174	0·4147	—	0·41605
K	0·03912	0·03998	—	0·03955
Ca	0·0163490	0·01638	0·01617	0·016299
Mg	0·006375	0·00542	—	0·005897
Cl	0·6224	0·6252	0·6189	0·62217

E. *The Serum of the Pollock.*—The solids in the serum of the pollock amounted in one determination to 7·095 per cent. The total salts in another estimation were 1·2934 per cent., and the Δ for the salts of this determination was −0·737° C., while the Δ for the serum was −0·825° C. The analyses of the ash gave:

	I.	II.	III.	IV.
Na	0·4133	0·4156	—	0·41447
K	0·01873	0·01721	—	0·01797
Ca	0·01198	0·01355	0·01306	0·01286
Mg	0·005686	0·00618	0·0064	0·00608
Cl	0·5626	0·55997	—	0·56128

IV. *Ratios of Values on the Basis of Na* = 100.

A. In Marine Invertebrates and in Sea Water.

	Na.	K.	Ca.	Mg.	SO_3.	Cl.
Ocean water[*] (Dittmar)	100	3·613	3·911	12·106	20·9	180·9
Limulus polyphemus[†]	100	5·62	4·06	11·20	13·33	186·9
Aurelia flavidula[‡] (Macallum)	100	5·18	4·13	11·43	13·18	185·5
Homarus americanus	100	3·73	4·85	1·72	6·67	171·2

[*] Calculated from Dittmar's analyses, '*Challenger* Report, Physics and Chemistry,' vol. 1.

[†] The earlier analyses of the blood of *Limulus* gave ratios (Na = 100) which are not in accord:—

	Na.	K.	Ca.	Mg.	SO_3.	Cl.
Gotch and Laws	100	5·30	3·83	11·67	7·65	157·8
Genth (*a*)	100	12·4	5·49	7·97	8·87	170·3
Genth (*b*)	100	6·11	4·86	11·06	8·56	161·5
McGuigan	100	4·72	5·51	10·29	4·83	168·5

Of these, as may be seen by comparison, Gotch and Law's results approach more closely those given by my analyses.

[‡] 'Journ. of Physiol.,' 1903, vol. 29, p. 213.

B. In Elasmobranchs, Teleosts, Mammals.

	Na.	K.	Ca.	Mg.	Cl.
Dogfish, *Acanthias vulgaris*	100	4·61	2·71	2·46	165·7
Cod, *Gadus callarias*	100	9·506	3·93	1·41	149·7
Pollock, *Pollachius virens*	100	4·33	3·10	1·46	137·8
Dog*	100	6·86	2·52	0·81	128·5
Mammal (average)*	100	6·69	2·58	0·80	118·3
Man†	100	9·22	3·37	1·76	101·4

* Calculated from Abderhalden's results, 'Zeit. für Physiol. Chem.,' 1898, vol. 25, p. 106.

† Calculated from the results of Bunge's analyses, 'Lehrbuch der Physiologischen und Pathologischen Chemie,' 1889, p. 221. The values for the K, Ca, and Mg appear high as compared with those obtained by me in analyses of the blood serum of man (unpublished).

V.—*General Observations.*

It will be noted on inspection of the results of the analyses that considerable differences exist between the blood of the Invertebrates, *Limulus* and *Homarus*, on the one hand, and the marine Elasmobranch and Teleost forms on the other.

In *Limulus* the amount of the total salts of the blood, 2·982 per cent., approaches that of the sea water, not indeed of the highest concentration, but of that which may be found at points along the Atlantic coast. At St. Andrews, New Brunswick, the total salts of the sea water collected in April were 2·417 per cent., but in sea water obtained in August 3·165 per cent.‡

In the blood of the lobster the total salts as ascertained were 2·852 per cent., which is between the two concentrations given above for the salinity of the sea water at St. Andrews, where the lobsters from which the blood was taken were obtained.

It is, however, when the ratios of the inorganic constituents based on the value Na = 100, as given above, are examined that one sees the close parallelism between the blood of *Limulus* and sea water. Only in the K and SO_3 are there important differences. The parallelism in ratios is all but complete between the blood of *Limulus* and the fluid in the disc of the Medusa, *Aurelia flavidula*. The Medusæ have probably always been marine forms, and *Limulus* and its ancestral prototypes have been oceanic as far back in geological history as the Cambrian. The parallelism in ratios not only between these forms but also between them and sea water, though striking, is not surprising. The blood of *Limulus* is but slightly modified sea water.

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‡ Macallum, "On the Inorganic Composition of the Medusæ, *Aurelia flavidula* and *Cyanea Arctica*," 'Journ. of Physiol.,' 1903, vol. 29, p. 213.

In the blood of the lobster the ratios, though they are on the whole parallel to those of sea water, differ from the latter, particularly in regard to the SO_3 and the magnesium. These are toxic constituents in sea water, and though in *Limulus*, because of its association since remote geological time with the ocean, a considerable degree of tolerance for them has been established, only a very limited adjustment to them has been developed in the lobster, whose marine history can be traced as far back as the Jurassic period only, that is to a far less remote period than in the case of *Limulus.* The ancestors of the *Homaridæ* are supposed to have been fresh-water forms of an astacoid character and a species of *Astacus*, *A. vectens*, is found in the so-called Lobster Beds in the Greensands of the Isle of Wight, belonging to the Cretaceous period. In the earlier period, the Jurassic, the macrouran decapods arose and their remains are found in deposits formed in more or less still waters, such as the Solenhofen slates, in which they are mingled with terrestrial and fresh-water forms. This suggests that the Mesozoic ancestor of the lobster of to-day must either have been of a fresh-water type or one that resorted to embayments, lagoons, or stretches of water more or less surrounded by land, and therefore of a fresh or slightly brackish character.

It is to be noted, further, that in the blood of the lobster the percentages of sodium and chlorine are 0·9033 and 1·547, while in *Limulus* they are 0·8885 and 1·6608. As the sea water at St. Andrews, New Brunswick, in April and August yielded on analysis 0·7423 and 0·9882 per cent. of sodium and 1·347 and 1·7473 per cent. of chlorine the sodium and chlorine concentrations in the blood of the lobster probably are approximately the mean values for the sodium and chlorine of its habitat. From this it would appear as if the sodium chloride of sea water passes freely into the blood till the sodium chloride concentration in both is approximately balanced.

In the serum of the dogfish, cod, and pollock the total salts are much less than in sea water. In the dogfish they are 1·7739 per cent., while in the cod and pollock they are 1·2823 and 1·2934 per cent. respectively. This marked difference is undoubtedly due to the difference in the length of time in which the Elasmobranchs and Teleosts have been associated with the ocean. The former have always been marine* since their origin in the Silurian period, while the Teleosts date only from the Jurassic and were probably derived from an *Amia*-like Ganoid. The Ganoids were abundant in Palæozoic and Mesozoic Seas, but these were probably also fresh-water forms, although through the scantiness of fresh-water deposits no evidence of such is known. The present day Ganoids, including *Amia*, are all fresh-water forms, and it is not improbable that the Ganoid ancestor of the Teleosts was a fresh-water form or one which, like the sturgeon, occasionally occurred in brackish water.

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* A shark, *Carcharias nicaraguensis*, is native of the fresh-water Lake Nicaragua.

The ratios of the sodium, potassium, calcium, and magnesium in the serum of these marine fishes are not on the whole very different and they approach those in the mammal. In the cod the potassium ratio (9·506) is high, and this may be due to a slight laking of the red corpuscles, which are rich in salts of that element. The ratio value for potassium in the pollock (4·33) is less than half of that in the cod, and it closely approximates that in the dogfish (4·61). The ratios in the dogfish and pollock are almost the same, and the difference is most marked only in the magnesium, which is more abundant in the former.

This excess in magnesium is apparently one of the results of the action of the sea water on the blood of the dogfish for all the time which has elapsed since the Vertebrate type arose, for the Elasmobranchs have, as already stated, been marine since their origin, which is, at the latest, Devonian and, if the Ostracoderms are Protoelasmobranch, probably early Silurian. The ratio for magnesium in the dogfish is, however, only a little more than one-fifth of that found in *Limulus*, which has been also since its origin in the Cambrian a marine form.

That the Teleosts have been oceanic for a much shorter time than the Elasmobranchs is shown in the osmotic pressure of their blood as measured by the Δ. In Elasmobranchs this varies somewhat with the species and the habitat, but from the determinations of Bottazzi,* Rodier† and others it has been found to lie between the values −2·03° C. and −2·44° C., while the sea water of the habitat of the animals, as a rule, gave a slightly lower Δ than the serum. In the dogfish the Δ was −2·035° C., of which the salts contributed −1·0737°. In the Teleosts it is always much less, rarely does it exceed −1°‡ and it ordinarily ranges between −0·466° in *Tinca vulgaris* and −0·838° in *Gadus virens*.§ In *Gadus morrhua*, according to Dekhuyzen, the Δ varied according to the locality from which the fish was taken and the minimum and maximum values were −0·644° and −0·811°. In the blood of *Gadus callarias*, as shown above, the salts gave a Δ of −0·71° C., while that of the sea water from which the examples of the cod were taken was −1·80°.* The blood in Elasmobranchs thus has an osmotic pressure approximately like that of sea water, while in Teleosts it is much less, in fact, only in excess of one-third. Dekhuyzen's observations would show that there is a tendency in the blood of Teleosts to increase the osmotic pressure with the increase in the saline concentration of the sea water, but it does not in *Gadus morrhua* pass beyond the limit of −0·811° C. The sea water thus influences only to a limited extent the osmotic pressure in Teleosts, while it has affected the blood in Elasmobranchs to the extent that the Δ is the same as or greater than it is in sea water. This balancing of the sea water and the blood plasma postulates an association with the sea for Elasmobranchs, which in duration exceeds enormously the time which has elapsed since the Teleosts arose. Strutt,† basing his observations on the amount of helium enclosed in a sample of hæmatite from the Eocene and on the amount of this gas liberated in a measured time from a certain quantity of uranium, suggests that about 30,000,000 years have passed since the Eocene. What then must have been the length of the interval between the Silurian and the present in order to account for the development of the high osmotic pressure in Elasmobranchs?

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* 'Arch. ital. de Biol.,' 1897, vol. 28, p. 61.

† 'Travaux des Lab. de la Soc. Scientif. et Station Zool. d'Arcachon,' 1899, p. 103. Reference in Hamburger, 'Osmotischer Druck und Ionenlehre,' vol. 1, p. 465.

‡ Bottazzi found the Δ in two specimens of *Charax puntazzo* to be −1·04° and −1·035°, and in a specimen of *Cerna gigas* it was −1·035°. Dekhuyzen questions these results as they were obtained with a cooling bath of −12° C.

§ M. C. Dekhuyzen, 'Kon. Akad. van Wetensch.,' Amsterdam, vol. 8, p. 537, 1905.

The difference between the Δ of the serum (−2·035°) and that due to the salts in it (−1·0737°) depends on urea and other organic solutes. Urea is present in large quantities in the blood of the Elasmobranchs. The first to note its presence in extraordinarily large amounts ("colossale Quantitäten"), not only in the blood but also in the muscles, liver, kidney, spleen, pancreas, ovaries, and testes of these animals were Staedeler and Frerichs,‡ who obtained as much as two ounces from the liver of a single shark (*Scyllium canicula*). Later, in 1890, von Schroeder§ found that in *Scyllium catulus* the blood as a whole had 2·6 per cent. urea. Assuming that the blood corpuscles were free from urea, the latter, he calculated, would constitute 3·1 per cent. of the plasma. In the liver and muscles of this animal it amounted to 1·36 and 1·95 per cent. respectively. Rodier,‖ also noted that one-third of the osmotic pressure of the blood of sharks is due to urea. As the Δ which he found in the blood of all the forms he examined amounted to −2·05°, as much as

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* The Δ of the sea water of the Atlantic along the coast of Nova Scotia and New Brunswick would seem not to exceed 1·90°. The sea water of Canso, the most eastern point of Nova Scotia, gave −1·825° and the maximum value for the sea water at St. Andrews, New Brunswick, was −1·85°.

† 'Roy. Soc. Proc.,' A, 1910, vol. 83, p. 96.

‡ 'Journ. für Pract. Chem.,' 1858, vol. 73, p. 48.

§ 'Zeit. für Physiol. Chem.,' 1890, vol. 14, p. 576.

‖ 'Travaux des Lab. Soc. Scientif. et Station Zool. d'Arcachon,' 1899, p. 103.

−0·68° was, therefore, due to urea, which must, consequently, be present to the amount of 2·18 per cent.*

The amount of urea in the blood serum of the dogfish was determined. The material which served for this purpose was that, portions of which had been used for the inorganic analyses detailed above. The serum had been preserved with thymol and was in good condition. Weighed quantities were mixed each with five times its volume of absolute alcohol, and the mixture held in a bottle placed in an agitator which was kept in motion for 24 hours. It was then filtered, the precipitate on the filter washed with absolute alcohol, and the combined filtrate and washings, after the volume was accurately ascertained, used for the determination of the urea. The method employed to this end was that of Folin and consisted in heating a measured quantity of the extract with magnesium chloride and hydrochloric acid for two hours, then adding strong alkali and distilling the liberated ammonia into standard acid solution, which was subsequently titrated with standard alkali. By this method the urea in four determinations as calculated from the ammonia found was: 1·965, 2·107, 2·017, and 2·017 per cent. The mean of these was 2·026 per cent.

This would give in the serum a lowering of the freezing point amounting to 0·63°. The latter with the amount of the depression due to the inorganic salts in the serum would total −1·7037°. As the Δ of serum is −2·035° there still remains −0·332° to be accounted for.

This is due to ammonia salts; although only infinitesimal traces of these were present in the alcoholic extract of the serum yet ammonia compounds were found in considerable amount in the serum itself. The explanation for this is that concentrated alcohol does not dissolve readily certain of the salts of ammonia, notably the phosphate,† and consequently, absolute or concentrated alcohol may be used to separate the urea and the ammonia salts in the blood.

The amount of ammonia in the serum of the dogfish was determined with the Folin method and the results of three estimations gave each 0·1727 per cent. of NH_3, or a concentration slightly greater than N/10. This would fully account for the depression −0·332°.

The high ammonia content, the extraordinary concentration of urea and the high percentage of salts, namely 1·7739, in the serum of the dogfish, all are the results of the action of the osmotic pressure of sea water on the blood of the dogfish, not for one or two geological ages but for all the time which has elapsed since the Cambrian period. The Teleosts, as pointed out above, arose in the Jurassic, and it is probable that the *Gadidæ* have been marine since the Cretaceous, yet in the blood of the cod the saline concentration is 1·2823 per cent. as against 1·7729 in the dog-fish. The difference, 0·49 per cent., would seem to be attributable to the longer life which the Elasmobranchs have undergone in the ocean, and it might be made the basis for determining the relative length of the time which elapsed between the Cambrian and the Cretaceous, could we with certainty know what was the original concentration of the salts in the blood plasma in the Protovertebrates of the Cambrian or Silurian. What it was we can only approximately conjecture.

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* Assuming that a gramme-molecular solution gives a Δ of −1·87°.

† Erwin Herter ('Mitth. Zool. Stat. zu Neapel,' vol. 10, p. 342) found the urine of *Scyllium catulus* rich in ammonia and P_2O_5 and it was markedly acid.

In the blood of mammals it is in the neighbourhood of 0·9 per cent.* The difference between this and the 1·282 per cent. in the serum of the cod, namely, 0·38 per cent., might be explained as caused by the action of the sea water for all the time since the Cretaceous. On this basis the length of the marine life of the *Gadidæ* would be to the length of the marine life of the Elasmobranchs as 0·38 is to 0·877.

It is, however, not well to base any views on these data. For all the length of time during which the *Gadidæ* have been associated with the ocean, the organic solutes in their blood must be very minute in quantity. The Δ of the serum and of its salts being respectively −0·765° and −0·71°, the difference (−0·055°) may be due to urea, ammonia salts, but even if due to urea alone the amount must be very small. The ratio between 0·055° and 0·63°, the Δ due to the urea in the dogfish blood, is very different from the ratio 0·38 : 0·877, and in consequence the latter cannot be regarded as indicating the relative durations of the oceanic history of the Teleosts and Elasmobranchs.

It may be that 0·9 per cent. is too low an estimate of the amount of salts in the blood plasma of the ancestral type of Vertebrate, and that just as in oceanic forms the growing saline concentration of the sea water tends and has tended ever to increase, though slowly, the salts of the blood, so in terrestrial forms the feeble salinity of their food and their environment may possibly have in the long ages decreased the salts of the plasma considerably below the ancestral standard. If the latter were 1·2 per cent., the increases in the salts of the serum in Teleosts and Elasmobranchs would be 0·08 and 0·57, and this would give the relative durations of oceanic life in these classes as 1 and 7.

It is impossible, however, to accept anything on this point as definite, and one may perhaps be in a position to speculate safely on such matters only when careful analyses have been made of the blood plasma of representatives of all the classes of Vertebrates, and specially of the fresh-water fishes.

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* According to Bunge (*loc. cit.*) the concentration of the salts in human blood serum is 0·842 0·867 per cent. Calculating from Abderhalden's analyses, the salts of the serum of the dog amount to 0·9354, of the cat 0·9331, and of the sheep 0·9053 per cent.

It is, nevertheless, certain that the inorganic salts have increased very slowly, much more slowly in the blood plasma of Elasmobranchs than they have in the sea water and, further, that the urea and ammonia salts have attained an extraordinary concentration. The explanation for this slow increase of the salts cannot be found in any inactivity of the epithelial cells of the mucosa of the intestinal tract, for Erwin Herter* found in the urine of *Scyllium catulus* 0·0415 per cent. Ca and 0·1416 per cent. Mg, as compared with 0·0464 per cent. Ca and 0·1421 per cent. Mg in the sea water of the locality (Naples) from which the animal was taken. The total amounts of the sodium and potassium were not determined, but the chlorine was estimated and found to be 1·3543 per cent., whereas in sea water at Naples it is 2·1142 per cent. This discrepancy may easily be explained, for the hydrochloric acid in the gastric juice in *Scyllium catulus*, according to Richet,† is from 0·69 to 1·29 per cent., and as the acid of the gastric juice is neutralised in the intestinal tract the chlorine thus contained may pass out with the intestinal excreta, and not by way of the kidneys, while its place in the renal excretion is taken by phosphoric acid, which is exceedingly abundant in the urine of this form. The correspondence between the sea water and the urine as regards the amounts of calcium and magnesium would seem to indicate very clearly that the intestinal tract, and, perhaps, also the gills, in absorbing fluids make little distinction, if any, between the water and the salts of the sea; in other words, sea water finds its way into the blood stream of the circulation in the Elasmobranchs.

It follows from this that it is the kidneys which determine the inorganic composition of the blood plasma in these forms. The kidneys not only regulate the total quantity of salts in the blood plasma, but they also maintain the ratios, almost as they obtain in higher Vertebrates, existing in the plasma between the sodium, potassium, calcium, and magnesium, even after long ages of exposure to the ever increasing saline concentration of the sea. That exposure, it is true, has had its result in increasing the total salts of the blood plasma, but the increase is but sufficient to bring the Δ due to them up to a little more than half of the Δ of the sea water of the habitat, or of the total Δ of the plasma.

It is in this respect that the dogfish differs completely from *Limulus*, although both they and their ancestral forms have been marine and contemporary almost throughout all the periods of geological history. In the king crab the renal organs do not influence the concentration of the salts of the blood, which amount to 2·982 per cent., and they appear to influence only extremely slightly the amount of the magnesium, and more considerably the sulphuric acid (SO_3). Even in the lobster, in which the saline concentration of the blood is 2·852 per cent., the renal organs are very active only in the elimination of these two elements.

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* 'Mitth. Zool. Stat. zu Neapel,' 1892, vol. 10, p. 342.

† 'Journ. de l'Anat. et de la Physiol.,' vol. 14, p. 170; also 'Comptes Rendus,' vol. 86, p. 676.

It is thus a far cry from the renal organ of *Limulus* and the lobster to the kidney of the Elasmobranch and still more so to the kidney of Teleosts and higher Vertebrates. The salts of the plasma of the cod are less than half those of the blood of the lobster, yet both *Gadidæ* and *Homaridæ* have been marine since the Cretaceous.

In mammals, according to Abderhalden's analyses, there is an extraordinary similarity in the inorganic composition of the serum of the number of the forms taken, and the ratios of the sodium, potassium, calcium, and magnesium are, as shown in Table (IV), B, almost parallel with those in the Teleosts and Elasmobranchs.

It may be of interest here to refer to the inorganic composition of the blood in mammalia which lead a marine life. Of these, the Cetacea have been marine since their origin in the early Eocene. So far no opportunity has occurred of analysing the serum of any of these forms, but through the kindness of Mr. G. W. Taylor, Director of the Canadian Marine Biological Station at Nanaimo, British Columbia, the author obtained a quantity of clotted and partially laked blood of the whale common in the Pacific off the coast of British Columbia. This was analysed and found to be very rich in potassium, as is the blood of the horse and pig, much the greater part of the element being held in the red corpuscles. The analyses gave the following values in per cent.:—

	Na.	K.	Ca.	Mg.	Cl.
1	0·1808	0·19962	0·00693	0·00444	0·26758
2	0·1802	0·20118	0·006005	0·00453	0·2695

If we take the mean of the two determinations of each element and range them with the values for the blood (corpuscles and plasma) of the horse and pig, as calculated from the determinations made by Abderhalden, the parallelism is remarkable:

	Na.	K.	Ca.	Mg.	Cl.
Whale …………	0·1805	0·2004	0·00646	0·00448	0·26854
Pig ……………….	0·17859	0·1917	0·00485	0·00533	0·2690
Horse ……………	0·19974	0·2273	0·00364	0·00384	0·2785

The fact that the inorganic composition of the blood in the whale is so like that of the pig* and horse strongly suggests that the inorganic composition of the serum is the same, although, almost as long as in the case of *Gadidæ*, the whales have been oceanic forms.

It would seem accordingly that in the power which the kidneys exercise of regulating and rendering uniform not only the saline concentration, but also the ratios of the sodium, potassium, calcium, and magnesium in the blood plasma of Vertebrates so far examined, we have to deal with an unalterable function of primal importance inherited from a Protovertebrate or Eovertebrate type of the Cambrian or even of the Pre-Cambrian.

The retention of urea and ammonia salts in the blood of Elasmobranchs undoubtedly developed as a result of the tendency of the blood to balance in itself the osmotic pressure of the sea water. The very fact that the kidneys in these forms exhibit some inertness in the elimination of urea, while they are very active in the elimination of the salts, is significant. What they do most rigorously is the regulation of the concentration and composition of the salts of the blood. *The firmly fixed physiological habit or function must be the more ancient one, and consequently the earliest function was not the elimination of waste metabolic products, but the regulation of the inorganic composition of the blood. The function of excreting waste products came later, and in the Elasmobranchs never acquired the fixity that characterised the function concerned in excreting the salts.*

That in the *Gadidæ*, although of marine habitat since the Jurassic, the kidneys rigorously keep down the saline concentration and regulate the inorganic composition of the blood, while the urea is readily eliminated, is not a difficulty in the way of accepting this view. The Ganoid ancestors of the Teleosts were fresh-water forms, probably throughout the latter half of the Palæozoic and, at least, the first half of the Mesozoic, and during all that time the conditions which would tend, as in the Elasmobranchs, to increase the osmotic pressure did not occur. There was nothing, then, to work against the elimination of waste nitrogenous matter, and this function, after the lapse of a long period of time, became fixed in the Ganoid kidney, with the result that, when the Teleosts arose, their kidneys had developed two very fixed functions, instead of one as in those of the Elasmobranchs, and these two have been transmitted with undiminished or slightly lessened force to their descendants, whether of fresh water or marine habitat, through the millions of years which have elapsed since the Jurassic. Thus, in the blood of the cod there is no accumulation of urea beyond the limit that is found in the blood of the higher Vertebrates.

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* The parallelism in composition between the blood of the whale and that of the pig and horse is so close that it is of special interest in connection with the origin of the Cetacea. Some anatomists relate them to the Ungulata; others question this on various grounds. The inorganic composition of the blood would seem to bring the whales very close to the Ungulates.

It is easy to understand how a uniformity of composition of the internal medium of animals is a powerful factor in influencing the course of evolution. The capacity of the organism to make and keep its own internal media uniform gives an enormous advantage to it, for it can change its habitat and adapt itself to a new environment without affecting the stable conditions under which its own tissues and organs do their best work.

This independence of external media is much more characteristic of Vertebrates than of any Invertebrates, and, in fact, it may be regarded as a special feature of Vertebrate life. It is, indeed, difficult to conceive how Vertebrates could have arisen and undergone the extraordinary development and adaptation to either terrestrial or aquatic life which they have experienced in geological time if their internal medium had not been maintained constant.

The establishment of that constant internal medium would therefore appear to have been the first step in the evolution of Vertebrates from an Invertebrate form. That, on the other hand, postulates that the kidney, developed to regulate and keep constant the internal or circulatory fluid, was essentially the first typically Vertebrate organ, and therefore of origin more ancient than that of the Vertebrate brain and spinal cord.

That to-day the earliest appearance in the Vertebrate embryo of structures which are subsequently to develop into the kidney is after the neural groove arises, constitutes, apparently, an objection to this view which can, however, be met. The distinctive parts of the ovary and testis of the Invertebrate form out of which the Protovertebrate developed undoubtedly gave rise to the distinctive parts of the ovary and testis in the Vertebrate. The reproductive cells of the ovary and testis are, therefore, of origin perhaps as remote in time as the origin of the Metazoa. In the Vertebrate embryo, however, the distinctively reproductive elements make their appearance at a date later than that at which the neural tube arises, and this retardation is, without doubt, due to the effect exercised by the postponement of the time when the sexual function begins to operate in the individual of a species. It may thus readily be that the renal organs, which do not function in the embryonic life of the individual, may arise relatively late, and yet in the Eovertebrate embryo have been amongst the very earliest structures to appear.

It is evident from the analyses of the blood of *Limulus* that the inorganic composition of its internal medium is determined by the composition of the ocean. If it were now to develop an excretory organ, with a function like that of the Vertebrate kidney, in its descendants in the far future, many millions of years from now, their internal medium, the blood, would in its inorganic composition reproduce in the main the ratios of the sodium, potassium, calcium, and magnesium, and also the saline content of the ocean of this age, although in that long interval the ocean would undergo very considerable change in composition and in saline concentration. The composition of the blood in that far remote future could be used to postulate the composition of the ocean of to-day.

So, from the composition of the blood plasma in Vertebrates, we may infer the relative composition of the ocean in the remote past, when the Vertebrate kidney acquired the function of controlling the salts and their concentration in the blood. This would give for the ocean of that age the ratios in round values as follows:—

Na.	K.	Ca.	Mg.
100	6·00	3·00	1·50—2·00

Compare this with the ratios of the ocean of to-day, which are:

Na.	K.	Ca.	Mg.
100	3·613	3·911	12·106

This would place the origin of the distinctively Vertebrate type, that is, of a form possessing a kidney with a function like that of the Vertebrate excretory organ, at a time when the ocean had one-eighth to one-sixth of its present concentration of magnesium, and less than one-third of its present content of sodium chloride.*

How far back in time that is to be placed cannot be estimated with certainty, for we do not know what was the sodium chloride content of the earliest ocean of the globe. Joly,† in calculating the age of the earth from the total sodium chloride now in the sea, and from the annual increment of the salt due to the river discharges from the land surfaces of the globe, assumes that it amounted to 14 per cent., or approximately one-seventh, that is, that the sea contained 0·4 per cent. of sodium chloride. If we accept this, it follows that, when the Vertebrate kidney began to evolve, the sodium chloride in the sea had increased only 0·3 per cent., and since then as much as 2·1 per cent. This would place the origin of the Eovertebrate form at a date not earlier than the second eighth, and not later than the first fourth of the whole geological period.

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* The concentration of sodium chloride in sea water is, in round numbers, 2·8 per cent., while in mammalian blood plasma it is about 0·7 per cent.

† "An Estimate of the Geological Age of the Earth," 'Trans. Roy. Dublin Society Series 2, 1899, vol. 7, p. 23.

We know, from the results of the analyses given above, that the magnesium in the blood of *Limulus* lags behind that of the ocean, which is ever growing in amount. The ratio between it and the sodium in mammal's blood may be put approximately 1 : 100. The ocean, then, in Eovertebrate time would have a higher magnesium content in relation to sodium, approximately 1·50–2·00 : 100, or one-eighth to one-sixth of the magnesium concentration of the ocean of to-day. These estimates also would place the origin of the Vertebrate kidney at a time somewhere between the beginning of the second eighth and that of the second sixth of the whole geological period.

These are speculations which are advanced with reserve. They may be accepted absolutely, or rejected wholly, only when we are in possession of the results of analyses of the blood plasma in all the representative Vertebrate classes, as well as of the blood of the higher types of Invertebrates.

Enough, however, has been advanced here to make it extremely probable that the inorganic composition of the blood plasma of Vertebrates is an heirloom of life in the primeval ocean.

The Anatomy and Morphology of the Leaves and Inflorescences of Welwitschia mirabilis.

By Miss M. G. Sykes.

(Communicated by Prof. A. C. Seward, F.R.S. Received May 28,—Read June 30, 1910.)

(Abstract.)

The paper deals with the anatomy of the leaves and inflorescences of *Welwitschia*, and with certain theoretical questions which arose during their examination. Cotyledons, leaves, bracts, and flowers each receive two bundles when young, though in the adult leaf numerous additional bundles are intercalated.

The transfusion tissue, which more or less surrounds the bundles in all the foliar members, is developed in the pericycle; in the bracts, and to a smaller extent in the old leaves, there is a further development of transfusion tissue from the cortical parenchyma. This second type of transfusion tissue recalls that of *Cycas*, and the presence of centripetal xylem at the base of the cotyledonary bundles is another character suggesting relationship with the Cycads.

The structure of the peduncles, cone-axis, cone-bracts, and flowers is essentially similar in both male and female inflorescences, which are therefore regarded as homologous.

In the naked inflorescence axes the formation of inversely orientated bundles and the presence of xylem elements in a position internal to the protoxylem again recalls the Cycads. Below the points of branching a series of elongated and waved bands of vascular tissue are formed, each consisting of a band of normally orientated xylem and phloem with an inversely orientated band on its outer edge. The structure at this level is compared with *Colpoxylon* and others of the Medulloseæ.

The two bundles which supply the female flower stalk each branch to form three; the central bundle of each trio passes out into the outer integument, while the remaining four give rise to the perinucellary ring, which dies out at the level at which the inner integument becomes free. From the position of this inner system it appears clear that it belongs to the inner integument.

In the male flower axis additional bundles are formed to supply the stamens, but the branching of the bundle is much as in the female. A

reduced perinucellary ring is formed at the base of the aborted ovule ; in it two large bundles are especially conspicuous, and probably represent the reduced vascular supply of the single integument, which is here regarded as representing the outer integument of the female flower.

The changes involved in the development of the fertile ovule consist chiefly in the growth of the region between the origins of the two integuments, and in the still greater enlargement of the region above the origin of the inner integument resulting in the formation of the remarkable nucellar beak. The micropylar tube or tip of the inner integument becomes strongly cuticularised. The embryo-sac extends from the level of the origin of the outer integument for some distance above the origin of the inner integument, and projects into the nucellar beak. Its position with regard to the two coverings thus corresponds roughly with the position of the embryo-sac in *Lagenostoma* in relation to the cupule and inner integument. The cupule is compared with the free outer covering of *Welwitschia*, and it is pointed out that the greater growth of the apical region of the Welwitschian ovule is probably correlated with siphonogamic fertilisation. The large nucellar beak provides also more space for the elongation of the embryo-sac and embryo, and may be regarded as the precursor of the well developed apical region of the nucellus in Angiosperms.

The seeds of *Cardiocarpus* and the Cycads are also compared with *Lagenostoma* and *Welwitschia*. In all these seeds the inner system of bundles appears to belong to the inner integument.

The ovule of *Welwitschia* also shows some points of resemblance to that of *Bennettites*. It is thought probable that the ovulate strobilus of the Bennettitals is comparable with a telescoped cone of *Welwitschia*, and that *Williamsonia angustifolia* represents an intermediate stage in the telescoping process.

It is concluded that *Welwitschia* is probably connected with the Cycadean series, with the Bennettitals, and with the ancestry of the Angiosperms. It seems remarkable that while both kinds of sporangia in the Cycads, the microsporangia in the Bennettitals, and probably both the ovules and stamens in the Angiosperms are foliar, the ovules of *Welwitschia* and *Bennettites* are commonly considered cauline. Possibly these also are primitively foliar, and long ago became separated from the leaf and inserted on the cone axis ; it is shown that the course of the bundles to some extent supports this suggestion.

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Page

THE EFFECT OF PRESSURE UPON ARC SPECTRA. No. 3.—SILVER; No. 4.—GOLD. By W. G. Duffield, D.Sc. (Abstract) 118

STUDIES OF THE PROCESSES OPERATIVE IN SOLUTIONS. XI.—THE DISPLACEMENT OF SALTS FROM SOLUTION BY VARIOUS PRECIPITANTS. By H. E. Armstrong, F.R.S., and J. Vargas Eyre, Ph.D. 123

THE DISTRIBUTION OF VELOCITY IN THE β-RAYS FROM A RADIO-ACTIVE SUBSTANCE. By J. A. Gray, B.Sc., 1851 Exhibition Scholar, University of Melbourne. (Plate 1) 136

THE DECREASE OF VELOCITY OF THE β-PARTICLES ON PASSING THROUGH MATTER. By William Wilson, M.Sc., Hon. Research Fellow of the University of Manchester 141

RATE OF EMISSION OF α-PARTICLES FROM URANIUM AND ITS PRODUCTS. By J. N. Brown, A.R.C.S. 151

ON RADIATION IN A GASEOUS EXPLOSION. By Bertram Hopkinson, F.R.S. 155

THE EFFECT OF SMALL TRACES OF WATER VAPOUR ON THE VELOCITIES OF IONS PRODUCED BY RÖNTGEN RAYS IN AIR. By Robert Tabor Lattey.. 173

ON THE VARIATION WITH TEMPERATURE OF THE VISCOSITIES OF THE GASES OF THE ARGON GROUP. By A. O. Rankine, D.Sc., Assistant in the Department of Physics, University College, London 181

ADDITIONAL REFRACTIVE INDICES OF QUARTZ, VITREOUS SILICA, CALCITE, AND FLUORITE. By J. W. Gifford 193

THE ACCUMULATION OF HELIUM IN GEOLOGICAL TIME. IV. By the Hon. R. J. Strutt, F.R.S., Professor of Physics, Imperial College of Science, South Kensington 194

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PROCEEDINGS
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BIOLOGICAL SCIENCES.

CONTENTS.

Page

The Assimilation of Nitrogen by certain Nitrogen-fixing Bacteria in the Soil. By W. B. BOTTOMLEY, M.A., Professor of Botany in King's College, London 627

On the Structure, Development, and Morphological Interpretation of the Pineal Organs and Adjacent Parts of the Brain in the Tuatara (Sphenodon punctatus). By ARTHUR DENDY, D.Sc., F.R.S., Sec. L.S., Professor of Zoology in King's College (University of London). (Abstract) 629

The Opposite Electrification produced by Animal and Vegetable Life. By W. M. THORNTON, D.Sc., D.Eng., Professor of Electrical Engineering in Armstrong College, Newcastle-upon-Tyne 638

The Fermentation of Galactose by Yeast and Yeast-juice. (Preliminary Communication.) By ARTHUR HARDEN, F.R.S., and ROLAND V. NORRIS 645

The Lignite of Bovey Tracey. By CLEMENT REID, F.R.S., and ELEANOR M. REID, B.Sc. (Abstract) 650

Obituary Notices of Fellows Deceased:—E. F. W. Pflüger; William Henry Dallinger; Thomas William Bridge . . . . . . i—x

Index xi

Title, Contents, etc.

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HARRISON & SONS, 45, ST. MARTIN'S LANE, LONDON, W.C.

Price One Shilling and Sixpence.

No. B 560.

September 23, 1910.

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The Assimilation of Nitrogen by certain Nitrogen-fixing Bacteria in the Soil.

By W. B. Bottomley, M.A., Professor of Botany in King's College, London.

(Communicated by Prof. J. Reynolds Green, F.R.S. Received June 20,—Read June 30, 1910.)

In a communication on "Some Effects of Nitrogen-fixing Bacteria on the Growth of Non-Leguminous Plants,"* it was pointed out that *Azotobacter* and *Pseudomonas* obtained from the root tubercles of Cycas when grown together fix more nitrogen per unit of carbohydrate than the combined amount of nitrogen when each is grown separately. In order to determine if this is true for a mixed culture of *Azotobacter* and *Pseudomonas* obtained from ordinary soil and leguminous nodules respectively, pure cultures of these organisms were obtained, *Azotobacter chroococcum* from garden soil and *Pseudomonas radicicola* from bean and clover nodules, by the method already described.* Erlenmeyer flasks containing a culture solution, consisting of maltose 0·5 gramme, mannite 0·5 gramme, monobasic potassium phosphate 0·1 gramme, magnesium sulphate 0·02 gramme, in 100 c.c. distilled water and rendered neutral by sodium hydrate, were inoculated with 1 c.c. of pure cultures per 100 c.c. of culture solution (the controls being autoclaved to kill the bacteria present), and incubated at 24° C. for 10 days. Nitrogen determinations of the contents of the flasks gave the following averages:—

Control	0·53 milligramme N in 100 c.c. per unit of carbohydrate.
Azotobacter alone	2·19 milligrammes N in 100 c.c. per unit of carbohydrate.
Pseudomonas alone	2·30 milligrammes N in 100 c.c. per unit of carbohydrate.
Pseudomonas + *Azotobacter*	4·51 milligrammes N in 100 c.c. per unit of carbohydrate.

Gerlach,† Lipman,‡ and others have described experiments showing that pure cultures of *Azotobacter* and *Pseudomonas* respectively have little or no power to increase the store of soil nitrogen when added directly to the soil. A probable explanation of these negative results may be found in the different conditions for growth and development in ordinary soil and in a culture solution.

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* 'Proc. Roy. Soc.,' B, vol. 81, pp. 287–289.

† 'Centralbl. f. Bakt. Abt. II.,' vol. 8, 1902.

‡ 'Reports New Jersey Expt. St.,' 1904—1907.

In order to induce the pure cultures to accommodate themselves to soil conditions, the following method was employed:—Some ordinary garden soil was treated with lime and sterilised in the autoclave. This was then thoroughly wetted with pure cultures of *Azotobacter* and *Pseudomonas*, and incubated at a temperature of 24° C. for 21 days. The organisms multiplied rapidly and spread through the soil, and adapted themselves to the special chemical and biological conditions to which they were subjected. Five grammes of this infected soil were mixed in 100 c.c. of water, to which 1 gramme of glucose had been added, and incubated for 24 hours. To test the effect of this culture solution in fixing nitrogen in the soil a series of five earthenware plant dishes were each filled with 5 oz. of rich garden soil which had been air dried and passed through a fine sieve to remove all stones, twigs, etc. Three of the dishes were limed—$\frac{1}{4}$ oz. of lime to each dish. The dishes were treated as follows:—

1. Watered with 50 c.c. distilled water.
2. Watered with 50 c.c. culture solution which had been autoclaved to kill the bacteria.
3. Watered with 50 c.c. living culture solution.
4. Watered with 50 c.c. distilled water.
5. Watered with 50 c.c. living culture solution.

The dishes were then placed in the incubator and incubated at 24° C. for 10 days, the soil being stirred with a sterile glass rod each day for aeration, and each dish receiving 50 c.c. of distilled water on the third, fifth, and eighth days of the experiment, to supply the loss of moisture due to evaporation.

The nitrogen determinations of the contents of the dishes gave the following results:—

1. Limed soil + distilled water	324 milligrammes N per gramme soil.
2. Limed soil + autoclaved culture	330 milligrammes N per gramme soil.
3. Limed soil + living culture	359 milligrammes N per gramme soil.
4. Unlimed soil + distilled water	327 milligrammes N per gramme soil.
5. Unlimed soil + living culture	352 milligrammes N per gramme soil.

Thus the mixed culture of *Azotobacter* and *Pseudomonas* gave an increase of 35 milligrammes of nitrogen on the limed soil, and an increase of 25 milligrammes of nitrogen on the unlimed soil.

This gain of nitrogen was not due to any material present in the culture solution, for the autoclaved culture solution shows a gain of 6 milligrammes of nitrogen only, derived chiefly from the dead bacteria in the solution.

Taking an acre of soil 4 inches deep as weighing about 1,000,000 lbs., a gain of 35 milligrammes of nitrogen per 100 grammes would represent an increase of nearly 350 lbs. of nitrogen per acre.

That the nitrogen fixed by this mixed culture of bacteria in the soil is readily assimilated by plants is shown by a number of experiments now in progress, full details of which will be described in a future communication.

On the Structure, Development, and Morphological Interpretation of the Pineal Organs and Adjacent Parts of the Brain in the Tuatara (Sphenodon punctatus).

By Arthur Dendy, D.Sc., F.R.S., Sec. L.S., Professor of Zoology in King's College (University of London).

(Received June 22,—Read June 30, 1910.)

(Abstract.)

The memoir of which an abstract is here given contains a detailed account of the pineal organs and associated parts of the brain in Sphenodon, from the morphological, histological, and embryological points of view, accompanied by numerous illustrations, and may be regarded as a continuation and amplification of my earlier work on the subject.

The material upon which my results are based consisted partly of a number of adult living Tuataras presented to me by the New Zealand Government, the cost of transmission of which to England was defrayed by a grant from the Government Grant Committee, and partly of specimens (chiefly embryos) preserved by myself while in New Zealand. I defer the expression of my thanks to the numerous friends who have helped me in the work until the publication of the complete memoir.

As I have already pointed out in my work on the intracranial vascular system,* there is in Sphenodon a very extensive subdural cavity between the brain and the cranial wall, and advantage was taken of this fact to fix the

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* 'Phil. Trans.,' B, 1909.

delicate organs of the pineal complex *in situ* by the injection into the cranial cavity of acetic bichromate of potash. After fixation the pineal eye itself, with the parietal plug, can be dissected clean out of the parietal foramen, and the necessity of decalcification before section cutting thus avoided.

The "pineal complex" is formed chiefly by the dorsal sac, the paraphysis and the pineal sac ("epiphysis" or right pineal organ), united in a common pial investment and forming a bilaterally flattened, funnel-shaped structure attached above to the cranial roof by the *dura mater* and below to the optic thalami and habenular ganglia.

Across the subdural space numerous fine threads of connective tissue extend from the surface of the brain to the *dura mater*, and these are concentrated to form imperfectly developed vertical supporting membranes for the pineal complex, one placed transversely on either side of the dorsal sac and one lying in the sagittal plane behind the pineal sac. The very large subdural space arises late in development, which perhaps indicates that the relatively small size of the brain is a cænogenetic character due to arrested growth.

The fundamental relations of the different parts of the fore-brain and its derivates in Sphenodon are already to a large extent familiar to us, but the following points may be noticed:—

Gisi has described the lateral choroid plexuses (*plexus hemisphærium*) as arising from a transverse fold behind the paraphysial opening, while Elliot Smith has described them as arising from a transverse fold in front of the paraphysial opening. The explanation of this discrepancy is that while they really arise one on either side of the paraphysial opening their roots of attachment may extend a little in front of and behind the latter. There is no unpaired *plexus medianus* as described by Gisi.

The paraphysis, as I have previously shown, is part of the same system of folds of the epithelial *lamina supraneuroporica* which gives rise to the *plexus hemisphærium*, but growing outwards instead of inwards. It originally opens into the prosencephalon immediately in front of the *commissura aberrans*. In an advanced stage of development, however, a longitudinal supra-commissural canal is formed above the *commissura aberrans*, and this leads to the formation of a new opening for the paraphysis in the adult, directly into the dorsal sac at some distance above the commissure. The original opening of the paraphysis, in front of the *commissura aberrans*, is blocked up by the growth of the anterior choroidal veins and arteries.

There is no true *commissura mollis* as described by Gisi, though the lateral walls of the third ventricle come into contact with one another over a considerable area.

Both in advanced embryos and in the adult animal three pairs of lateral diverticula, in addition to the cerebral hemispheres, the optic lobes, and the pineal outgrowths, open into the central canal of the fore- and mid-brain. These are, from in front backwards, (1) the *recessus optici laterales,* which appear to be remnants of the cavities of the stalks of the optic vesicles; (2) a pair for which I propose the name *recessus thalami prenucleares,* because they lie in the substance of the optic thalami in front of the *nuclei rotundi;* (3) the *recessus geniculi* (of Gisi), which lie on either side of the entrance to the *iter*, beneath the posterior commissure. It is suggested that these three pairs of diverticula may be serially homologous with one another and with the cerebral hemispheres, the outgrowths which form the pineal sense-organs and the optic lobes, and that each of these pairs of outgrowths indicates an original neuromere. In accordance with this view the cerebral hemispheres would belong to the first neuromere of the fore-brain, the optic vesicles of the lateral eyes to the second, the *recessus thalami prenucleares* to the third, and the pineal outgrowths to the fourth, while the *recessus geniculi* would belong to the first, and the optic lobes to the second neuromere of the mid-brain.

The middle portion of the pineal complex is formed by the thin-walled dorsal sac, the roof of which gives rise to a well-developed choroid plexus supplied by branches of the saccular arteries. The folds of this choroid plexus are covered with an epithelium composed of polygonal cells with well-defined boundaries and conspicuous nuclei. Attached to this epithelial layer, and lying in the cavity of the dorsal sac between the folds of the choroid plexus, is a cytoplasmic network containing numerous nuclei and apparently composed of extrusive connective-tissue cells. The choroid plexuses of the fourth and lateral ventricles are practically identical in histological structure with that of the dorsal sac.

The paraphysis grows upwards immediately in front of the dorsal sac, and its upper end turns backwards over the roof of the latter. It must be regarded as a compound tubular gland. Its walls become greatly folded, and in the adult we find a central lumen surrounded by numerous crypts and opening into the dorsal sac. Between the crypts numerous blood spaces develop, which sometimes form a regular network of thin-walled sinuses or capillaries. These are supplied with blood by paraphysial branches of the saccular and anterior choroidal arteries, and drain into the *sinus longitudinalis* beneath the pineal sac.

The paraphysis is invested by *pia mater*, which attaches it firmly to the dorsal sac. Its epithelial lining has a very characteristic histological structure, consisting of a single layer of cells without distinct boundaries, and connected together by radiating threads of cytoplasm to form a syncytium. In connection with this epithelium there is a very conspicuous but irregular network of nucleated cytoplasm lying in the paraphysial lumina. The nuclei in this network are very poor in chromatin and undergo amitotic division. Sometimes little rounded knobs, covered with the syncytial epithelium, project from the wall of the paraphysis into its various cavities.

I have already described the origin of the two pineal organs from the brain-roof, and how, from its first appearance, the one which is destined to give rise to the pineal eye usually lies a little to the left of the other, which will give rise to the pineal sac. I am now able to confirm Schauinsland's subsequent observation that these two vesicles are at first in open communication with one another, but I do not consider that this need prevent us from regarding them as members of an originally symmetrical pair, and fresh evidence in favour of this view is put forward in the present memoir.

The opening of the pineal sac into the third ventricle, between the superior and posterior commissures, closes up at a comparatively early date, but vestiges of the connection remain in the "infra-pineal recess" and in the "pineal tract" by which the pineal sac of the adult remains connected with the brain-roof. The pineal sac grows upwards in close contact with the posterior wall of the dorsal sac, to which it is firmly attached by the *pia mater*, and its upper end turns forwards over the roof of the dorsal sac and over the upper part of the paraphysis. In the adult it is a relatively large organ and takes an important part in the formation of the pineal complex. It remains tubular, but its walls become greatly folded and much thickened. They are supplied with blood by the anterior and posterior pineal arteries and drain into the *sinus longitudinalis*. There is little or no evidence that the pineal sac is a glandular body, but, on the contrary, its histological structure points to a sensory function. Its thick wall is made up of nucleated radial supporting fibres, numerous ganglion-cells and nerve-fibres, and numerous sense-cells whose inner ends project slightly into the lumen of the organ. These constituents are identical with those which occur in the retina of the pineal eye, and their arrangement is essentially the same. In one case, in which the tip of the pineal sac projected unusually far forwards, so as to come under the influence of the light passing through the transparent parietal plug, a pigmented evagination of the wall of the pineal sac was formed, and the resemblance to the retina of the pineal eye became still more obvious. These observations, confirming and extending earlier observations by Hoffmann, Gisi, and myself, greatly strengthen the view that the pineal sac and pineal eye are bilaterally homologous structures.

The pineal sac is provided with a well-developed nerve, composed of non-medullated fibres, which runs in the "pineal tract" and joins the brain-roof in the middle line between the superior and posterior commissures, which remain perfectly distinct throughout life.

The histological structure of the pineal eye itself has been investigated with especial care, and various methods of fixation and staining have been employed for the purpose. The sharp distinction between lens and retina appears at a very early date, and though they remain in contact with one another throughout life, the actual connection between the two is henceforth very slight, and the transition from the one to the other is perfectly abrupt.

At a very early stage the development of the nerve-fibres divides the retina into a thick inner and a thin outer layer, with the nerve-fibre layer between them. The inner layer contains many nuclei belonging to sense-cells, and also nuclei which belong to ganglion-cells. The outer layer contains only a single layer of nuclei, belonging to the radial supporting fibres. Later on the ganglion-cells come to lie more to the outside of the nerve-fibre layer, next to the nuclei of the radial fibres.

In the adult retina, omitting for the moment the pigment, we find only three kinds of histological elements: (1) radial supporting fibres, (2) ganglion-cells and nerve-fibres, (3) sense-cells.

The radial supporting fibres are comparable to the Müller's fibres in the lateral eyes, and probably extend right through from surface to surface of the retina, their inner ends forming the well-developed internal limiting membrane, and their outer ends abutting against the inner capsule of the eye. Their nuclei appear to be all lodged in their outer portions, which have the misleading appearance of a layer of short conical cells.

The ganglion-cells are numerous, and are readily distinguished by their large spherical nuclei, finely granular cytoplasm (with usually one large projection), and the shrinkage cavity which surrounds them.

The sense-cells are slender, elongatedly spindle-shaped, with large oval nuclei. Their outer ends run into the layer of nerve-fibres. Their inner ends project slightly into the cavity of the eye, but are covered with little conical caps, formed apparently by extension of the internal limiting membrane.

In most respects the structure of the retina agrees closely with that of Anguis and Lacerta as recently described by Nowikoff. That author, however, gives a somewhat different account of the projecting ends of the sense-cells and of the distribution of the pigment.

Baldwin Spencer considered that in Sphenodon the pigment was especially associated with the sense-cells; Nowikoff, on the other hand, maintains that in Anguis and Lacerta the pigment is lodged in the radial supporting fibres. According to my own observations on Sphenodon, the pigment granules lie between the various constituents of the retina, and are brought in from outside the eye by wandering pigment cells. Such cells are abundant in the connective tissue around the eye, between the inner and outer capsules, and sometimes they also occur in the form of pigment-balls in the cavity of the eye itself, having apparently passed through the retina without breaking up and discharging their contents. Usually, however, they appear to break up in the outer part of the retina, and the granules which they contain stream in in radial lines and streaks between the radial fibres and sense-cells, to such an extent as greatly to obscure the histological structure of the retina. The wandering pigment-cells may possibly obtain their pigment granules from the very large stellately branched pigment cells which lie in the *dura mater* outside the capsule of the pineal eye.

The pigment is especially abundant towards the margins of the retinal cup, near its junction with the lens, and here accessory cavities are not infrequently developed in the retina, each surrounded by radiating streaks of pigment granules. The lens contains only occasionally a very few pigment granules. The vitreous body also usually contains very little if any pigment, but occasionally a good deal.

At stage R, when the pigment first appears, it is found only in very minute granules, chiefly, if not entirely, in the inner part of the retina. In the adult much coarser granules appear, though the small ones can still be recognised in the innermost part of the retina.

Perhaps the most novel results obtained are those which concern the lens of the pineal eye, which is shown to be a glandular organ, secreting part, at any rate, of the vitreous body. At a very early stage in development we can recognise two zones in the lens, an outer or marginal zone, in which the cells remain undifferentiated and continue to divide actively by mitosis; and a central portion in which the cells become greatly elongated at right angles to the two surfaces of the lens, which thereby becomes greatly thickened in the middle. Growth of the lens is probably effected mainly by the marginal zone of actively dividing cells, but it is not impossible that the cells may continue to divide after elongation. The distinction between the central and marginal zones of cells persists to a very late stage in development, though possibly not in the adult.

In the adult the arrangement of the elongated cells becomes far less uniform, and they are irregularly curved so as to appear cut through in various directions in vertical sections. They probably extend right through from surface to surface of the lens, but their inner ends are somewhat specially differentiated, and project as small rounded protuberances into the cavity of the eye. The nuclei are situated at various levels, and the cytoplasm of the inner portions of the cells is very distinctly fibrillated in a longitudinal direction, while darkly staining bodies resembling centrosomes can sometimes be seen close to the inner extremities of the cells.

In the adult lens, about the middle, one usually, if not always, finds one or more irregular masses of a finely granular, deeply staining substance. It was the observation of a large mass of this kind, with a centrally placed nucleus, which led to my description of a "central cell" in the lens, and it was chiefly with a view to further investigation of this remarkable structure that this research was undertaken. I now find that such central masses are very constant features of the adult lens, and their true nature was indicated by the fortunate occurrence of an adult specimen in which such a mass was actually being extruded in the form of "mucus" into the cavity of the eye to take part in the formation of the vitreous body. I have observed this extrusion of "mucus" into the cavity of the eye in several cases, and as early as stage R. With the "mucus," nuclei may pass out from the lens, and there can be no doubt that the secretion is formed by degeneration of cells in the middle of the lens. The extrusion always appears to take place from the middle of the lower surface of the lens at a very definite spot, but an actual aperture is probably present only at the time when the secretion is being poured out.

The vitreous body always contains, in preparations, a reticulum of slender fibres or thin lamellæ, and some of these are attached, on the one hand, to the inner surface of the lens, and, on the other, to the inner surface of the retina, apparently in many cases to the projecting ends of the sense-cells, but probably really to the caps which cover these. Whether the presence of this reticulum is due to *post-mortem* changes or not remains an open question.

A large amount of time has been devoted to following out the course of the nerve of the pineal eye, and I have been able to demonstrate very clearly that it is not a median structure, but belongs to the left side of the body—another striking piece of evidence in favour of the paired origin of the pineal organs.

In the adult animal the anterior end of the nerve, like the eye itself, has been shifted into the middle line. For the greater part of its course, however, it lies between the wall of the pineal sac and the wall of the dorsal sac, and considerably to the left side of the middle line. It is very easy to follow it from the eye towards the brain up to a certain point, where it breaks up into a number of separate strands. This point lies between the posterior wall of the dorsal sac and the anterior wall of the pineal sac, not far from the lower extremity of the latter. Up to this point it consists of a well-defined bundle of non-medullated nerve-fibres, with a definite sheath of connective tissue in its more anterior portion, and with numerous elongated nuclei lying between the nerve-fibres. It exactly resembles an ordinary non-medullated nerve, and I can see no reason for regarding it as exhibiting degeneration. The separate strands into which it breaks up at the point mentioned, however, do not contain the characteristic elongated nuclei, which doubtless really belong to associated connective-tissue or nutrient cells, and owing to the slenderness of these strands, and the difficulty of distinguishing them from the connective-tissue fibres of the *pia mater*, I have not succeeded in following them continuously to the brain in the adult animal.

In several series of sections of embryos of different ages, however, the nerve has been traced to the brain as one continuous bundle of fibres without difficulty, and it is quite clear that it enters the left habenular ganglion. It becomes closely attached to the roof of the dorsal sac, however, before it reaches the habenular ganglion—or the spot where this will be developed—and this fact probably explains why the lower part of the nerve is broken up into separate strands in the adult, for the rapid growth of the thin wall of the dorsal sac may be supposed to cause the spreading out of the nerve-fibres over its surface.

Nerve-fibres first appear in the retina of the pineal eye while the latter is still resting upon the brain-roof, and I have come to the conclusion that they grow from the retina to the brain as in the case of the lateral eye.

A curious feature of the nerve of the pineal eye in the adult animal is that it receives bundles of nerve-fibres from the wall of the pineal sac as well as from the eye itself. This point has already been noted by Gisi.

The left habenular ganglion in the adult is produced upwards to meet the wall of the dorsal sac in a characteristic manner at a point where it receives nerve-fibres from the latter in special abundance. The right habenular ganglion also receives fibres from the wall of the dorsal sac, but is not produced upwards to the same extent as the left one. This asymmetrical development of the habenular ganglia further supports the conclusion that the left habenular ganglion is especially associated with the pineal eye.

It is extremely difficult to form any conclusion as to how far the pineal eye of Sphenodon still functions as a light-percipient organ. Such experiments as have hitherto been made have yielded entirely negative results. The concentration of a bright light upon the skin above the pineal eye elicits, so far as I have been able to make out, no response; but then it must be remembered that the animals are extremely sluggish, and a similar experiment with the lateral eye may be continued for some time without producing any visible effect beyond the contraction of the iris.

Structurally, the only sign of degeneration which the pineal eye exhibits is to be found in the very large amount of pigment present in it in the adult, for I do not think we need regard the degeneration of the central lens-cells into the mucus which helps to form the vitreous body as of any significance in this respect.

Eigenmann has shown that a great deal of pigment is developed in association with the degenerating lateral eyes of the blind fishes, Lucifuga and Amblyopsis, but the degeneration of the pineal eye of Sphenodon does not approach in degree that of the lateral eyes of these types, and there seems no reason why it should not still function as a light-percipient organ. The formation of images by the lens is, of course, out of the question, on account of the irregular arrangement of the small scales which overlie the parietal foramen. I find from direct experiment, however, that light can pass through the integument at this point, and also through the more or less transparent parietal plug which covers the pineal eye in the foramen.

Reissner's fibre and the sub-commissural organ ("ependymal groove") are well developed in Sphenodon, and appear to have the usual relations. The latter has the form of a deep groove, lined by the characteristic greatly elongated columnar epithelial cells, and extending forwards from near the hinder end of the posterior commissure, beneath the latter, to the infra-pineal recess. Reissner's fibre is already conspicuous at stage S.

The Opposite Electrification produced by Animal and Vegetable Life.

By W. M. Thornton, D.Sc., D.Eng., Professor of Electrical Engineering in Armstrong College, Newcastle-upon-Tyne.

(Communicated by Dr. A. D. Waller, F.R.S. Received June 17, 1910.)

1. *Introductory.*—When a steady electric current is passed through a drop of pond scum rich in animal and vegetable organisms, two opposite movements of migration of the living cells will in general be observed. Diatoms and unicellular algæ, for example, move towards the negative pole, amœboid animal organisms to the positive. The clearness of the effect is often confused by the presence of anchored and skeleton cells of either kind, and the case of pond scum is only given because the effect to be described was first observed in this way.

Early in 1904 it was desired to find an indicator for the qualitative detection of voltage gradient in liquids in fields of microscopic dimensions. The orientation of long diatoms into line with the current was anticipated from the known behaviour[*] of bacteria in an alternating field, and found to occur. In steady, that is unidirectional, fields they not only orientate but move along the line of current-flow to the negative pole. They therefore serve in two ways to indicate the presence of a steady current in the liquid.

At the first, and in all subsequent trials, the movement of migration of the diatoms was accompanied by one in the same direction of portions of most of the vegetable matter free to move, and a simultaneous movement in the reverse direction of the free animal forms, provided that the motile activity of the latter did not overpower the mechanical influence of the field. The movement was quite dead-beat, reversing instantly with the reversal of polarity, and since the only mechanical force an electric field can exert is upon an electric charge it was concluded that the opposite movement of the cells indicated that they carried opposite charges, the animal cells being negatively electrified, the vegetable cells positively.

2. *Method of Experiment.*—To observe the movement it is well to have the field as strong as possible; it was found that 75 volts per centimetre was the highest which could be conveniently used. This is much greater than that employed by previous workers, which rarely exceeded 10 volts per centimetre.

To avoid trouble by liberation of gas at the electrodes with this high gradient it is necessary to use a small current, that is to have a high resistance between the poles, such as one obtains from a thin film of liquid of high resistivity, 300 to 1000 ohms per centimetre cube.

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[*] L. Lortet, 'Comptes Rendus,' April 20, 1896, p. 892.

The method used was to place a drop of tap water, weak saline or cane sugar solution, on a glass slide, to introduce by a platinum needle the cells to be observed, and to place a $\frac{3}{4}$-inch square cover slip over it. The drop was just sufficient to fill the space below the slip without flooding it.

The electrical pressure was then applied from a hand generator of the kind used in testing the insulation of electric cables, giving about 150 volts when turned slowly, 500 volts quickly, on open circuit. The pressure from a direct-current electric lighting supply with a water resistance or bank of lamps in series also serves well. The current strength should be such that bubbles form at the electrodes very slowly or not at all. The current was led into the drop by means of two fine platinum wires secured to an insulating cross-bar by wax, and attached by flexible wires to a reversing key and the generator. The bar could be raised or lowered freely. The stand holding it was of such a height that the wires were almost horizontal, lying along opposite sides of the cover slip. Using a $\frac{1}{12}$-inch oil-immersion lens the cells crossed the field in a few seconds when the generator was turned slowly.

It is difficult when working with bacteria to avoid the influence of streaming under the slip caused by change of capillarity through electrification, and it is only by repeated trial that the exact thickness of liquid for dead-beat movement can be obtained. With blood and yeast cells, however, the electro-mechanical force on the cell is much greater, and streaming gives less trouble. Its occurrence can in any case be readily seen by the organisms continuing to move after the stoppage of the current.

Before dealing with living cells it must be stated that almost all finely divided matter can be made to migrate in an electric field in suitable liquids. This has been very fully investigated, notably by Quincke,* Hardy,† Perrin,‡ and Burton.§

The present results are differentiated from these by the clear division between the movement of the two types of life, in general irrespective of habitat, culture medium or composition of the cell, provided that this is fresh. The intensity of the movement is conditioned largely by the relative conductivities of the organisms and the surrounding liquid.

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* Wiedemann, 'Electricität,' vol. 2, pp. 166 *et seq.*

† W. B. Hardy, 'Roy. Soc. Proc.,' B, 1900, vol. 66, p. 110.

‡ Jean Perrin, 'Journ. de Chim. Physique,' 1904, vol. 11, 10, p. 607.

§ C. V. Burton, 'Phil. Mag.,' April and November, 1906.

In repeating the experiments the chief difficulties will be found to be (1) in maintaining electrical contact between the wires and the drop without excess of liquid; (2) in the cells adhering to the slide and cover slip; (3) in distinguishing between forced movement of the cells and free movement due to streaming. Whenever there is no movement either the electrical circuit is broken or the cells are anchored. To avoid the disturbing influence of ions from the poles the current should be reversed every five to ten seconds of continued observation.

The essential points are fresh cells, neutral liquid, high voltage and resistance; in addition to which the electrical conductivity of the liquid should be less than that of the organisms. The reason for the latter is that when it is greater the current avoids the cell, preferring to go by the liquid. On the other hand, when the conductivity of the organism is greater the current converges upon it.

3. *Infusoria.*—From time to time attempts were made to obtain conditions under which the effect could be demonstrated with certainty. Motility of any kind gave trouble; thus *Paramœcium* was found by Verworn[*] to move to the negative pole; but if the current is switched on while clusters of the organisms are under observation, they will be found almost without exception to burst towards the positive. This was also observed by Verworn. *Euglena*, again, bursts towards the positive. The cell contents of Rotifers and of small fresh-water worms move towards the positive, the sacs clearing on the negative side. Many similar observations were made, but the results were, as a rule, not sufficiently marked or uniform for repeated demonstration. These were all with cells in their natural habitat. Dale,[†] by a careful examination of the movement of parasitic infusoria in various solutions, arrived at the following results. The reaction of the host—a frog—was always alkaline. In an alkalinated solution *Opalina ranarum* moved to the positive (p. 310); *Nyctotherus cordiformis* from a freshly killed frog to the positive (p. 316); *Balantidium entozoon*, alkaline, in strong currents to the positive, then later to the negative (p. 321); *Balantidium elongatum* (p. 326), and *Balantidium duodeni* (p. 332), first to the positive and after some hours to the negative. It is not stated whether the organisms were then found to be living, but is of interest to note that the movement, whilst they were fresh, was in each case to the positive. The reversal may be compared with that of plants given in the next section. In acid solutions the movements were in general reversed, but the

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[*] 'Psycho-physiologische Protistenstudien,' Jena, 1889.

[†] H. H. Dale, 'Journ. of Physiology,' vol. 26, pp. 219 *et seq.*

conditions are then not those of normal life or growth, since the reaction of the fresh frog was alkaline.

Starting from Hardy's conclusion (*loc. cit.*) that acid particles are electrically negative and basic particles positive, Lillie* investigated the movement of certain animal cells. He found that the red corpuscles and smaller leucocytes—in frog's blood—move to the positive, the voluminous leucocytes to the negative. With spermatozoa there is active migration of the sperm head to the positive, the tail having been absorbed in the cane sugar solution used. With teased-out tissue the effect was not so certain, which he suggests may be the result of *post-mortem* alteration of muscle substance, and of the injury in teasing out the cells. His conclusions are "that the direction and speed of electrical migration of living cells and portions of tissue are chiefly dependent upon the electrical characteristics of their constitutent colloids, that animal cell nuclei exhibit a strong tendency to migrate to the positive pole, and that this is strongest in those nuclei in which the proportion of nucleic acid is highest."

Perrin (*loc. cit.*), discussing colloidal solutions, observes that "a charge which is raised by the presence in the solvent of a monovalent acid is lowered by a monovalent base," an extension of Hardy's conclusion. Jennings'† paper on "The Reactions of Electricity in Unicellular Organisms" is a criticism of others by Birnkoff and Greely on the migrations of infusoria, chiefly *Paramœcium*. Greely states that, in his opinion, "the electrical condition of the protoplasm itself determines the motion."

4. *Vegetable Cells.*—In the present experiments the first clear movement of vegetable matter observed, other than that of diatoms, was of filaments of *Vaucheria*, which moved to the negative. *Volvox aureus* moved and burst to the negative, as was also observed by Carlgren.‡ *Sphærella plantaginis*, a pleurococcus (chlorophyll green alga), a unicellular alga, and protococci from moist growths, all moved to the negative. Working by the method described, the following bacteria, when taken from young active growths and examined immediately, moved without exception to the negative:—*B. typhosus*, *B. tuberculosis*, *B. diphth. avium*, *B. prodigiosus*, B. Lactic acid, *B. pyocyaneus*, *B. coli comm.*, *B. Friedländer*, *Sarcina aurantiaca*, *Sarcina lutea*, *Staph. aureus*, Spore-bearing bacillus, Hog cholera, Pneumococcus. Bacteria from cultures which had been standing in the laboratory for some time and were not sub-cultured before being examined, and bacteria which, though sub-cultured 24 hours before use, showed very poor growth, almost invariably moved to

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* R. S. Lillie, 'Am. Journ. of Physiol.,' 1903, vol. 8, p. 276.

† H. S. Jennings, 'Journ. Neurology and Psychol.,' 1905, pp. 528—534.

‡ Archiv f. Anat. a. Physiol.,' Physiol. Abth., 1900, p. 49.

the positive. This reversal is no doubt accompanied by marked changes in the protoplasm. In the case of an unclassified non-motile bacillus, in the laboratory of the University of Durham College of Medicine, isolated from a scarlet fever patient, a most active migration to the negative was obtained, which, tested by samples from the same agar tube kept after incubation in a cool place, lasted for about ten days, when it reversed. No further observations were possible on this organism because it soon after died out.

Russ,* working with weak voltage gradients, small currents and many hours' exposure, has recently found some bacteria to migrate to the positive pole, others to the negative. In view of the present experiments the former may be attributed to either motile response to stimulus, or the forced movement of organisms of which the charge had been reversed by prolonged action of the solution.

With regard to the movement of growing plants, Brunchhorst† found that roots extending into water through which a moderate current was passed bent towards the negative, but when the current was strong to the positive. He regarded the latter effect as secondary and due to electrolytic action. Ewart and Bayliss‡ regard it as stimulatory. They apparently agree that, with moderate currents, the movement to the negative is a response to stimulation.

Jost§ notes that "roots which in the strong current exhibit positive curvature always die, not only on the positive side but altogether, at the latest after 24 hours. The negative curvature appears to be genuine stimulation, with the root apex acting as the organ of perception." Letellier|| also observed curvature of growing roots towards the negative pole. The sporangiophore of *Phycomyces* was found by Hegler¶ to bend towards the negative pole under the influence of Hertzian radiation.

From the difficulty experienced in working with motile organisms it was decided that although there was cumulative evidence that the difference between the animal and vegetable reaction was real, a crucial test could only be made with non-motile cells of either kind, the animal cells fresh from the animal, the vegetable cells from an active growth.

5. *Blood Corpuscles and Yeast.*—Human blood corpuscles satisfy the conditions in every way. They are readily obtained fresh and, containing a high percentage of salts, are not too sensitive to external influences. At first it

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* C. Russ, 'Roy. Soc. Proc.,' 1909, vol. 81, p. 314.

† 'Ber. d. bot. Ges.,' 1884, p. 207.

‡ 'Roy. Soc. Proc.,' B, 1905, vol. 77, p. 63.

§ 'Plant Physiology' (trans. Harvey Gibson), 1907, p. 481.

|| 'Bull. de la Soc. bot. de France,' 1899, vol. 6, p. 11.

¶ 'Verhandlg. d. Ges. deutscher Naturf. Aerzte in Halle,' 1891.

was thought that bacteria could be found to satisfy the conditions equally well, in spite of the difficulty of observing them readily, but in any loopful there are liable to be inert organisms which confuse the result. Many observations were, however, made with blood corpuscles and bacteria mixed in a drop of tap water or weak saline solution. When the latter were fresh the effect was marked, the blood cells of both kinds moving actively to the positive, the bacteria to the negative, streaming past one another, stopping, starting, and reversing with the current.

For the purpose of demonstration the difficulty was to find a vegetable growth of great vigour with single non-motile cells of convenient size for observation. This was eventually found in *Torula.* Yeast is easily obtained and complies with all the requirements. Blood and yeast cells are of the same size, but the slight colour of the former and the oval shape of the latter enable them to be readily distinguished. Judging by the velocity of migration the charge carried is of the same order in each. In a field containing many of both kinds they move past each other and after collision pass on with unchanged speed. The effect can be readily projected upon a screen with arc light, using a No. 6 objective well stopped down, the disc obtained being about 3 inches in diameter. It is unlikely that the electrical charges reside only on the surface, for they would, at least in part, coalesce, as they do in neutralised colloidal solutions.* The charges of blood and yeast cells appear also to be exceedingly stable. Smears of either on glass, dried for several days, on being moistened with water exhibit the effect quite well.

It may then be reasonably concluded, in so far as it is possible to have a single crucial test for so wide a range of activities, that fresh animal cells are negatively, and vegetable cells positively electrified. The fact that collisions do not discharge the cells, and that in both kinds the reversal of charge (which has no doubt led to most of the conflicting evidence on electrical migration of cells) takes place slowly, and cannot well be located in the cell wall, suggests that the above conclusion may tentatively be extended to the protoplasm. The principal difference between the two modes of life would then be that active animal protoplasm produces negative electrification, vegetable positive. Expressed in terms of Hardy's results the former is acidic, the latter basic in type.

6. *Contributory Evidence.*—This conclusion is not without support from less direct experiment. It has been shown by Dr. Waller† that the local skin response current is opposite in sign in animal and vegetable tissues, indicating an essential difference in the normal charge of their cells.

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* W. B. Hardy, *loc. cit.*

† A. D. Waller, 'Signs of Life,' p. 84, § 51.

From experiments on the action of static charge on growing plants Lemström* found that air positively ionised stimulated growth much more than when the charge was negative.

These observations agree with the view that there is an essential difference in sign between animal and vegetable charge, that of vegetable growth being positive.

If then a supply of negatively charged matter raises the activity of animal protoplasm and depresses that of bacteria, it may be even possible in disease to stimulate the blood directly by the inhalation of negatively charged air. It is worth examining where in nature air is found to have a free negative charge. Elster and Geitel† have shown that at high altitudes there is always a much stronger discharge of negative than of positive electrification. The action of sunlight, especially of the ultra-violet rays, is to cause a leak of negative charge from growing leaves and from pine trees in particular, the latter no doubt in part from the discharging influence of sharp points. The presence of negative charge in high pine woods from a combination of these causes may account in some measure for their marked curative properties, in tuberculous disease of the lung for example. In view of the difficulty of attacking tubercle *in situ* and the importance of the desired result, the suggestion is offered that the inhalation of air charged artificially with negative ions might prove useful in the treatment of tubercle of the lung, either by raising locally the activity of the blood cells or lowering that of the organisms.

It may be remarked that nascent oxygen and chlorine, which in electrolysis carry a strong negative charge, are active bactericides, hydrogen, the only electropositive gas, is not.

7. *Classification of Rudimentary Organisms.*—When the cells are fresh or active the described effect provides a sensitive means of distinguishing between animal and vegetable. Thus *Lycogala* (Mycetozoa) moved to the positive pole and was thus, in the specimens examined, animal in type. Resting spores of *Badhemia utricularis*, after several hours in water, moved equally well to the negative pole, as vegetable. Whether the latter observation is an example of the reversal of sign of an animal cell, similar to that constantly found with bacteria, or an indication that the organism may possibly be vegetable in type, requires further examination. Mr. J. J. Lister‡ observes that "*Badhemia utricularis* is exceptional in feeding on living fungi, though it will also live and thrive on the same fungi after they have

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* S. Lemström, 'Electricity in Agriculture,' 1904, p. 62.

† 'Ann. d. Physik,' 1900, vol. 2, p. 425.

‡ 'A Treatise on Zoology' (ed. Sir E. Ray Lankester), "The Protozoa," p. 49.

become dried, if they are wetted again with water." It also differs from many Mycetozoa in growing in strong sunlight.*

It would be of interest to examine in this way the rudimentary organisms which at present occupy an undefined position between the two great divisions of living matter. If protoplasm is identically the same in animal and vegetable cells the principle of life for each must be the power of determining its activity so that as a result the charge of one becomes negative and the other positive. It is also possible that the protoplasm itself may not be the same, and that the characteristic electrical charge is obtained not by separation within the cell but by selective absorption from without, so that the protoplasmic cell-content cannot be regarded as quite the same in the two cases.

In conclusion I wish to express my indebtedness to my colleague, Prof. H. J. Hutchens, for most useful advice.

The Fermentation of Galactose by Yeast and Yeast-juice. (Preliminary Communication.)

By Arthur Harden, F.R.S., and Roland V. Norris.

(Received July 18, 1910.)

(From the Biochemical Laboratory of the Lister Institute.)

Numerous investigators have shown that many species of yeast which ordinarily do not ferment galactose, readily acquire that property when cultivated in a medium containing that sugar.

The present communication briefly describes experiments which have been made with living yeast in this way, and with the juice obtained from this yeast. Experiments are also proceeding with yeast "killed" by acetone and other reagents (zymin), and it is hoped when these are complete to give a more detailed account of the whole investigation in a future paper.

Pure cultures of S. Carlsberg I have been used throughout these experiments. The medium employed for "training" the yeast consisted of yeast water to which was added 20 per cent. of hydrolysed lactose and 0·15 per cent. of K_2HPO_4.

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* *Loc. cit.*, p. 48.

Kahlbaum's galactose was used and the last traces of glucose removed by fermentation with ordinary brewers' yeast. The experiments were all carried out at 25°, those with yeast-juice being performed in the presence of toluene.

I. *Action of Untrained S. Carlsberg I on Galactose and Glucose.*

For this experiment wort cultures were employed and quantities of 1 gramme of the yeast were incubated with solutions of galactose and glucose and with water, the volume being the same in each case. The galactose was not fermented at all.

Table I.—Untrained S. Carlsberg I on Galactose and Glucose.

Experiment.	Time, in minutes.	Total fermentation. C.c. of CO_2 evolved in the time given.		
		Galactose.	Glucose.	Autofermentation.
1	65	2·5	52·9	2·4
2	35	1·2	—	1·3

II. *Fermentation of Galactose and Glucose by S. Carlsberg I grown in a Medium containing Hydrolysed Lactose.*

The results tabulated below show that the ratio of the rates of fermentation of these two sugars varies between wide limits. The conditions controlling this have not yet been ascertained.

Table II.—Comparison of Rates of Fermentation of Galactose and Glucose by S. Carlsberg I after Cultivation in a Medium containing Hydrolysed Lactose.

Experiment.	Mean rate of fermentation for 30 minutes. C.c. of CO_2 evolved in 5 minutes.		Ratio $\frac{\text{Glucose}}{\text{Galactose}}$.
	Galactose.	Glucose.	
3	2·81	2·35	0·84
4	10·21	19·25	1·86
5	1·52	2·32	1·52
6	2·52	3·52	1·39
7	4·00	6·63	1·65
8	1·65	2·18	1·31
9	1·37	3·50	2·55
10	2·70	7·60	2·83
11	5·35	15·00	2·79

III. *Fermentation of Galactose by Yeast-juice.*

The juice was obtained from S. Carlsberg I cultivated in the medium described above. The yeast was well washed with water and then ground in the usual manner.

Experiment 12.—Two quantities of 20 c.c. juice, to one of which 1 gramme of galactose had been added, were incubated at 25° in presence of toluene.

Experiment 13.—Three quantities of 25 c.c. of a different juice were incubated as above; to one was added 2 grammes galactose, to the second 2 grammes glucose, and to the third 1 c.c. H_2O.

Table III.—Fermentation of Galactose by Yeast Juice obtained from Trained S. Carlsberg I.

Experiment.	Fermentation in 1 hour. C.c. of CO_2 evolved.		
	Galactose.	Autofermentation.	Glucose.
12	49·0	8·4	—
13	39·3	8·6	25·7

These experiments clearly show that galactose is readily fermented by the juice obtained from yeast which has acquired the property of fermenting this sugar.

IV. *Total Fermentation of Galactose and Glucose by Yeast-juice.*

Experiment 14.—Two quantities of 25 c.c. of the same yeast-juice as used in Experiment 13 were incubated with 2 grammes galactose and 2 grammes glucose respectively in presence of toluene.

The initial rate of the galactose solution was higher than that of the glucose, but the total volume of gas evolved was slightly greater in the case of glucose. Fermentation had ceased in both cases after 19 hours.

Table IV.—Total Fermentation of Galactose and Glucose by Yeast-juice from Trained S. Carlsberg I.

Initial rate. C.c. in 5 minutes.		Total fermentation.		Ratio of totals.	
Galactose.	Glucose.	Galactose.	Glucose.	Galactose.	Glucose.
3·7	3·1	94·9	99·2	0·95	1·00

V. *Fermentation of Galactose by Yeast-juice in presence of Phosphate. Relation of extra Carbon Dioxide evolved to Phosphate added.*

The addition of phosphate to a fermenting mixture of galactose and yeast-juice produces an acceleration in the rate, and an extra amount of carbon dioxide is evolved which is equivalent to the phosphate added. As in the case of glucose, the phosphate is converted into an organic compound which cannot be precipitated with magnesium citrate mixture. The compound formed can be isolated in the form of its lead salt as described by Young for glucose.*

The extra carbon dioxide was evolved much more rapidly in the case of glucose than with galactose with the particular sample of juice employed.

Experiment 15.—Two quantities of 25 c.c. yeast-juice were incubated with 1 gramme of the sugar under investigation until a constant rate was obtained and 2·5 c.c. of a 0·3 molecular solution of sodium phosphate then added to each.

Table V (*a*).—Acceleration of Rate of Fermentation of Galactose and Glucose by Yeast-juice on addition of Phosphate.

Experiment 15.

Time after addition of phosphate, in minutes.	CO_2 evolved in preceding 5 minutes.	
	Galactose.	Glucose.
(Rate previous to addition)	0·8	1·3
5	5·3	8·3
10	3·6	7·9
15	3·4	5·7
20	2·8	2·6
25	2·4	1·9
30	2·5	1·3
35	2·1	1·3
40	2·0	
45	1·8	
50	1·5	
55	0·9	

Table V (*b*).—Equivalence of extra CO_2 evolved to Phosphate added in above Experiment.

	Galactose.	Glucose.
Maximum rate obtained	5·3	8·3
Final rate	0·9	1·3
Total gas measured	29·1 in 1 hour	27·7 in 30 mins.
Correction for rate without phosphate	10·8	7·8
CO_2 due to phosphate	18·3	19·9
,, at N.T.P.	16·7	18·2

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* 'Roy. Soc. Proc.,' B, 1909, vol. 81, p. 528.

The phosphate added was equivalent to 16·7 c.c. of CO_2 at N.T.P. in the ratio $Na_2HPO_4 : CO_2$.

The phenomena observed with galactose are therefore precisely the same as those exhibited by glucose.

VI. *Formation of an Organic Phosphate.*

25 c.c. of juice + 2 grammes galactose were incubated and phosphate added in small quantities until no further acceleration could be produced.

When the rate had fallen to its original value, the free phosphate in the boiled and filtered liquid was estimated.

Free phosphate found was equivalent to	0·1400 gramme $Mg_2P_2O_7$.
The phosphate added corresponded to	0·3953 „ „

Hence phosphate equivalent to 0·2553 gramme $Mg_2P_2O_7$ has been converted into a form not precipitable by magnesium citrate mixture. The constitution and properties of this compound are under investigation.

VII. *Fermentation of Galactose by Yeast-juice in Presence of Arsenate.*

As with glucose the addition of small quantities of sodium arsenate to a fermenting mixture of galactose and yeast-juice produces an acceleration, but hitherto it has only been found possible to double the rate in this way.

Conclusions.

1. The statement by various workers that some yeasts can be trained to ferment galactose by cultivation in a medium containing that sugar, has been confirmed.
2. Such a trained yeast yields a juice capable of fermenting galactose.
3. A fermenting mixture of yeast-juice and galactose reacts with phosphate in a similar manner to yeast-juice and glucose. The rate is accelerated, an extra amount of CO_2, equivalent to the phosphate added, is evolved and the rate then again becomes normal. The phosphate is converted into an organic compound not precipitable by magnesium citrate mixture.
4. The fermentation of galactose by yeast-juice is also accelerated by the addition of small quantities of sodium arsenate.

The Lignite of Bovey Tracey.

By Clement Reid, F.R.S., and Eleanor M. Reid, B.Sc.

(Received May 30,—Read June 16, 1910.)

(Abstract.)

In 1863, Heer and Pengelly published in the 'Phil. Trans.' an account of these lignite-beds and their flora. Heer classed the lignite as Lower Miocene, considering it equivalent to the Aquitanian of France and to the Hamstead Beds of the Isle of Wight. These latter are now referred to the Middle Oligocene, and many of the other deposits called Lower Miocene in Heer's day are now classed as Upper Oligocene.

A statement by Mr. Starkie Gardner, that Heer's Bovey plants are the same as those found in the Bournemouth Beds (Middle Eocene), has caused the Bovey Beds to be classed as Eocene in recent text-books and on recent maps of the Geological Survey, leaving a great gap in the geological record in Britain. Every division, from Upper Oligocene to Upper Miocene, was supposed to be missing.

Our recent researches have not supported this view, but tend rather to show that Heer was right—the Bovey lignite is highest Oligocene, or perhaps lowest Miocene. We could not find in the Bournemouth collection (now in the British Museum) anything to support Gardner's view, and he does not appear to have collected at Bovey, his comments referring to the collection now in the Museum of Practical Geology. We therefore made a collection of seeds and fruits in the Bovey deposits, as far as the flooded state of the lignite-pit would allow, in order to settle if possible the true age of the lignite.

The results were unexpected, for by using new methods we obtained a considerable number of species, and they were mainly identical with well-known plants of the lignite of the Wetterau, which is generally classed as Upper Oligocene. In certain cases, better specimens showed also that Heer's supposed peculiar species of Bovey belong to well-known forms of the Rhine lignite—his *Vitis britannica*, for instance, is only a crushed seed of *Vitis teutonica*. Several curious new species were discovered, including the earliest known *Rubus*, a peculiar *Potamogeton*, and a new genus of *Boragineæ*.

A study of the anatomy of the cone and leaf of *Sequoia couttsiæ* proves that it is a true *Sequoia*, not, as has been suggested, a species of *Athrotaxis*.

OBITUARY NOTICES

OF

FELLOWS DECEASED.

CONTENTS.

Page

E. F. W. Pflüger i

William Henry Dallinger iv

Thomas William Bridge vii

E. F. W. PFLÜGER, 1829—1910.

EDUARD FRIEDRICH WILHELM PFLÜGER was born at Hanau-on-Main on June 7th, 1829, and died at Bonn in April, 1910, in his eighty-first year. Although his career appears to have been singularly uneventful, it is important as a long record of untiring physiological research. He had few interests outside of the laboratory where he spent the greater part of his active and strenuous life. To those who knew him but slightly, it comes rather as a surprise to hear that he was married and had children (four daughters), for of no one can it be more truly said that he was "wedded to his work."

He originally studied law in Berlin, but he soon went over to medicine; it was while engaged in qualifying himself as a practitioner of this art that he came under the influence of Johannes Müller and Emil du Bois-Reymond, and the work he performed under the direction of these two great masters initiated the series of physiological investigations which has made his name famous.

In 1855 Pflüger obtained his doctorate, three years later (1858) he was "habilitiert," and in 1859 was appointed Professor of Physiology in the University of Bonn, in which chair he succeeded Helmholtz, who was called to Heidelberg. He was still holding this post at the time of his death, for although he was offered the Chair of Physiology at Berlin after du Bois Reymond's death, he declined it; 1878 was the year in which the new Institute of Physiology at Bonn was opened, and the next "red-letter day" in his life was 1909, in which year he celebrated his eightieth birthday and the jubilee of his appointment as Professor. He was elected a Foreign Member of the Royal Society in 1888.

The famous journal of physiology which he founded, and which soon became familiarly known as Pflüger's Archiv, was bound up with Pflüger's life, and it was here that he published nearly all of his work; he was its most voluminous contributor, and 130 volumes had appeared before he died. It rapidly became in Germany the physiological journal which had the largest circulation, and, in consequence, there was never any lack of contributions. Pflüger, however, was a careful editor, and had to reject many of the papers sent in to him. He was exacting in the manner authors set out their matter, and they were bound to obey his rules on such small matters as to the way in which they inserted their references, if they wished to get their papers printed. This untiring diligence lasted to the end, and he was correcting proof sheets as he lay in bed until within a few days of his death.

On the occasion of his jubilee, Nussbaum, one of the most celebrated of Pflüger's former pupils, compiled a complete list, which reached to some hundreds, of the papers Pflüger had written. Some of these are world-famous, but many no doubt had been forgotten even by those whose duty it is to study current bibliography. What strikes one most on glancing through the long array, is the catholicity of Pflüger's tastes; hardly any corner of the wide physiological field was left untouched; papers on histology, physiological chemistry, physical physiology, metabolism, embryology, psychology, and even philosophy are included, and he seldom touched a subject without illuminating it.

Pflüger is perhaps best known for his epoch-making work on muscle and nerve, and Pflüger's law, as it is called, is familiar even to the junior student. His work on metabolism, where he entered the lists against Voit, of Munich, is equally worthy of his repute, and although no one believes now in the theories of either of these two extremists, Pflüger had the great merit of recognising and of making others recognise the importance of the cell in the changes which food material undergoes before it is ultimately disposed of. The substance glycogen always attracted a peculiar fascination for Pflüger, and the importance of his work in relationship to this material is universally acknowledged. In the later years of his life, his most numerous and his longest monographs dealt with this subject, either from the chemical point of view or from the physiological aspect of its origin and fate.

Pflüger gathered around him a band of younger workers, and conveyed to them much of his own enthusiasm. He set them to work at various subjects in which he was interested, and often important investigations were the result. Much of the work which issued from the Bonn laboratory on the blood-gases was initiated in this way. Many of his pupils and colleagues became his friends and enthusiastic admirers; nevertheless Pflüger could not have been an easy man to get on with; he would not brook any interference with his work and his ideas, and the differences in the laboratory more than once were followed by the appearance of acrimonious articles and the severance of association in work.

The combativeness of Pflüger's nature and his love of polemics constituted a feature which was not wholly admirable in his character. Professor Cyon, however, who has recently written in Pflüger's Archiv an appreciation of his master, is inclined to regard this characteristic as a sign of his strength; he tells us that Pflüger prided himself on his militant attitude, and regarded controversy as the surest means of eliciting the truth on disputed problems. One may also freely admit that although in such disputes Pflüger tenaciously maintained his views, he was not so prejudiced as to continue to hold them when he was convinced of the truth of the opposite opinion. For instance, through a long series of papers, the results of most laborious researches performed even down to details of quantitative analysis by his own hands, he maintained that glycogen does not originate in the body from protein material. Yet in one of his very latest papers he had to surrender this position, because still further work had convinced him he had been wrong.

As the years of life advance, very few escape that conservatism and distrust of new ideas that mark the veteran thinker. Perhaps Pflüger had as little of this failing as any other man of the same age, but it was nevertheless there.

His attack on the neuron doctrine, and more recently on the work of Emil Fischer, are cases in point. Those interested in the knowledge of protein chemistry, which had its inception in Fischer's remarkable work, are hoping that by a continuation of that work one of the most difficult of biochemical questions will shortly be solved. The paper from Pflüger's versatile pen is certainly entitled to the fullest respect and consideration, but we must remember that Pflüger many years ago had a theory of protein constitution which is now of historical interest only; his views on the work of Fischer, Abderhalden and their disciples are therefore not unexpectedly pessimistic, for he doubts whether their gigantic array of experiments brings us any nearer to a solution of the problem. Pflüger adopted as the only certain test for a protein its capacity to maintain life and enter into the composition of protoplasm. If such a definition is accepted, gelatin, protamines, the whole of the poisonous proteins, the proteoses and polypeptides must be excluded from the protein family. This definition strikes one as too narrow and too biological; it is quite possible that members of a group may possess chemical characters in common which justify the use of a general name, and yet they may have a very different physiological action. This is admitted for substances of which the chemical constitution is known, and it is no great stretch of the imagination to conceive the same to be true of substances which, like the proteins, are still in chemical darkness.

But an obituary notice is not the place for a discussion of difficulties of this kind. Much of what Pflüger did will certainly stand the severest of all tests, that of time, and his name will be handed down as one of the giants of his era. His courage and love of truth, his devotion to science, made him an example which may well be imitated, and the scientific men of this country will unite with the whole world in mourning with Germany the loss of one of her greatest sons.

W. D. H.

WILLIAM HENRY DALLINGER, 1842—1909.

William Henry Dallinger, the son of an artist and engraver, was born at Devonport on July 5, 1842. As a boy he had leanings towards natural science and at one time had thoughts of becoming a medical student; but the deep piety of his nature prevailed and, after a brief training at Richmond Theological College, he entered the Wesleyan ministry in 1861, and for the next twenty years "travelled in the circuits" of Faversham, Cardiff, Bristol, and Liverpool. At the last-named city he remained for twelve years. During these years he not only kept up his interest in Natural Science, but taught himself German, Greek, and Hebrew.

In 1880 Dallinger was appointed Governor and Principal of Wesley College, Sheffield, where he did much to develop the modern side of the school. There he remained until 1888, when the Wesleyan Conference, in recognition of the great interest and value of his scientific work, allowed him to retain the status and privileges of a minister without pastoral charge, only retaining his position as a member of the "Legal Hundred." On leaving Sheffield, Mr. and Mrs. Dallinger—he married Emma, daughter of David Goldsmith, of Bury St. Edmunds, and had one son—were presented with some plate and a handsome sum of money, which he characteristically spent in microscopes and other scientific instruments.

During his tenure of the Principalship of Wesley College, Dallinger was four times elected President of the Royal Microscopical Society, in 1884-5-6 and 7. Although living 160 miles from London, he was constant in his attendance at meetings both of the Council and of the Society, and in order in no way to allow these meetings to interfere with his work at Sheffield he was in the habit of returning by the early newspaper train the morning after the Meeting. His devotion to the Society and the tact he showed in the Chair were warmly commented on by Dr. Glaisher, Prof. Jeffrey Bell, and Mr. Crisp on his retirement in 1888. So great was his interest in the Society that shortly after resigning the Presidency he allowed himself to be nominated Joint Secretary, and for some years he continued to labour whole-heartedly for its welfare. In 1890, 1891, and 1892 he was President of the Quekett Microscopical Club.

After leaving Sheffield, Dallinger devoted much of his time to lecturing. He was for many years senior Lecturer of the Gilchrist Educational Trust. He first lectured for the Trust in 1879 and continued without a break until the spring of 1909. During the thirty years he gave about 450 lectures in different towns in the country for the Gilchrist Trustees. The titles of some of his most popular lectures were as follows:—

"An Hour with the Modern Microscope," "Carnivorous Plants," "Contrasts in Nature—the Infinitely Great and the Infinitely Small," "Evolution as Illustrated in the Minutest Forms of Life," "Spiders: Their Work and Their

Wisdom," "Ants," "Wasps," "The Pond and its Minute Inhabitants." In 1887 he was chosen to deliver the seventeenth Fernley Lecture, the Lectureship being a Wesleyan foundation. The lectures are delivered at the annual Conference. Dallinger took as his subject "The Creator, and what we may know of the Method of Creation." As a lecturer Dallinger was very successful: he had a vivid descriptive style and a remarkable ability in illustrating both verbally and by drawings his subject matter. He spared no pains to make his matter attractive and even painted his own lantern slides; in this his remarkable artistic gifts were apparent.

Some of his more important scientific articles are mentioned below. The great service he did to students by editing and partly re-writing Carpenter's book on "The Microscope" is worthy of record.

Dallinger was elected a Fellow of the Royal Society in 1880, and received the degrees of LL.D. from Victoria University, Toronto, in 1884; D.Sc. from Dublin in 1892; and D.C.L. from Durham in 1896. A striking photograph of him is published in the 'Journal of the Royal Microscopical Society,' 1909.

The original contributions made to Science by Dr. Dallinger were almost entirely confined to the investigation of certain flagellates. In conjunction with his friend, the late Dr. J. Drysdale, of Liverpool, he succeeded in working out the life-history of many of the minuter forms and of making considerable advances in our knowledge of the processes of decomposition of organic matter, and of the degree to which unicellular animals can survive great changes in the temperature of their environment. The dominant feature of the work of Dallinger and his colleague Dr. Drysdale was untiring patience and unwearied application combined with a profound knowledge and mastery over all the technique of the microscope. By using a binocular, one individual flagellate could pass from the vision of one observer to that of the other and thus on one occasion a motionless zygote was continuously watched for thirty-six hours until it burst into a cloud of swarm spores; on another occasion Dallinger watched the same protozoon for a continuous period of nine hours. By such persistent observation the life-histories of several of the flagellates hitherto most incompletely known were worked out. The papers in which the joint authors recorded the observations are characterised by a singular modesty and simplicity of language. The simple organisms investigated were not overwhelmed with long classical designations. *Dallingeria drysdalei* (S. Kent) was to them "the hooked monad," *Polytoma uvella* (Ehrb.) "the acorn monad," *Tetramitus rostratus* (Pertz) "the calycine monad," and so on.

Dallinger and Drysdale contributed important facts bearing on the theory of "abiogenesis." With great manipulative skill and under the most rigorous conditions they were able to show that though the temperature of boiling water is fatal to flagellates in an active state, the spores of these animals can resist a much higher temperature without suffering harm. For these minute spores can sustain a heat up to 268° F. in water, and even up to 300° F. or higher if in a dry state. The demonstration of these remarkable powers of resistance showed that organic solutions, which had been thought to be sterile because they had been boiled, often contained living spores which had survived the heat and were capable of starting fresh colonies of flagellates. In connection with this part of his scientific researches was the remarkable series of experiments by means of which he was able to habituate successive generations of *Dallingeria drysdalei* and other forms to gradually increasing higher temperatures. From a temperature of 60° F. at which these flagellates normally live, he gradually raised the solution to that of 158° F. At this heat, which is at once fatal to the normal animals not habituated to high temperatures, the animals lived and multiplied, differing from the original stock chiefly in the marked vacuolation of the protoplasm. He felt that these experiments weighed against the position Weismann had taken up on the non-inheritance of acquired characters, and argument did not shake him. He was intensely preoccupied in his work and gave his contemporaries the impression of profound earnestness in all he undertook, combined with a little absence of business method. Before leaving this short record of Dallinger's contributions to the advancement of learning it is well to recall the honest and the truly scientific spirit which animated both his researches and his writings. This is admirably expressed in his own words :—" Let *truth* come from whence it may, and point never so grimly to where it may, he would be recreant to science who would for one moment hesitate to receive it. But no less false is it to the foundation principles of true science, to accept as true, what must constitute the roots of vast generalisations, except on evidence which no future scrutiny or analysis can shake."*

A. E. S.

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* 'Jour. of the Roy. Mic. Soc.,' vol. 3, pt. 1, p. 16 (1880).

THOMAS WILLIAM BRIDGE, 1848—1909.

Thomas William Bridge, the eldest son of the late Thomas Bridge, was born in Birmingham on November 5, 1848.* He received his early education at the Moseley School, and later attended science classes at the Midland Institute in Birmingham. In November, 1869, he became private assistant to Mr. J. W. Clark, then Superintendent of the University Museum of Zoology at Cambridge, and now Registrar of the University. He did not matriculate until 1871 and he entered Trinity College as a Foundation Scholar in 1873. A Demonstratorship of Comparative Anatomy having been established in the University in the latter year, Bridge was nominated to the post by the late Prof. Newton, his duties consisting in conducting a practical class in Comparative Anatomy, in addition to his work in the Museum. We are informed in the Annual Report of the Museums and Lecture Rooms Syndicate for 1873 that his class was well attended and that his pupils derived much profit from his instruction.

After graduating by means of the Natural Sciences Tripos (1875), Bridge spent six months at the Zoological Station at Naples. The outcome of this visit was the paper on the "Pori abdominales of Vertebrata." Returning to Cambridge, he again took up his duties as Demonstrator, and was engaged as before in teaching and curatorial work. The Cambridge Museum still possesses many admirable dissections, particularly osteological preparations of Fishes, which were prepared by him at this time and earlier.

In February, 1879, he was appointed, in succession to Dr. Leith Adams, F.R.S., to the Professorship of Zoology in the Royal College of Science for Ireland, vacating it a year later on his election to the Chair of Biology at Mason College, Birmingham, then just about to be opened. In 1882 he became the first Professor of Zoology in Mason College on the division of Biology into Zoology and Botany; and with the development of that institution into a University in 1900 he became Mason Professor of Zoology, a position he held until his death.

From 1880 onwards, as in his early life, Bridge's interests were entirely in Birmingham. His official duties naturally occupied a large proportion of his time, and his connection with a young and expanding institution rendered these claims so exacting as to give him but little leisure for research. It was no doubt mainly owing to this cause that the period between 1878 and 1888 was unproductive of scientific results. But he took a full share during that time in the organising work incidental to the growth of Mason College, acting as Secretary to the Academic Board in 1884—1886, and in later years occupying successively the Vice-chair and the Chair of that body.

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* For many of the facts and dates recorded in this notice the writer is indebted to Miss Bridge and to an article contributed to the 'Birmingham Post' by Prof. J. H. Poynting, F.R.S.

To his professorial work he throughout gave the utmost devotion. He was an excellent lecturer, and took special interest in the practical work carried on by his students.

Bridge was closely connected with the Birmingham Natural History Society and Philosophical Society, of both of which he filled the office of Vice-President, becoming the first President of the amalgamated Societies in 1894. He proceeded to the degree of Sc.D. (Cambridge) in 1896, and became a Fellow of the Royal Society in 1903. The degree of M.Sc. was conferred on him by the new University of Birmingham in 1901.

Bridge's scientific work all lay within a narrow compass. He was essentially a Morphologist, and his original papers refer to Fishes, especially to those which are usually regarded as occupying a low place in the Piscine series. He was thus particularly attracted to the "Ganoids" (a name which is now used in a somewhat more restricted sense than that in which he was accustomed to use it), to the Dipnoi and to the Siluroids, *Osteoglossum* and *Notopterus* among the Teleostei. "Let it be distinctly understood that the only sound foundation for scientific ichthyology is a profound *comparative* anatomy, and especially osteology of all the genera." These words, by Dr. T. Gill,* well express what may be supposed to have been Bridge's guiding motive throughout his work, which was always a judicious mixture of description and comparison of the structure of well-selected forms of Fishes.

During his residence at Cambridge he took up, jointly with his friend Mr. A. C. Haddon, the study of the remarkable relations that exist between the air-bladder and the auditory organ in the Siluridæ, as in certain other families of Teleostei which are grouped together as Ostariophysi. This resulted in a paper published in the 'Proceedings of the Royal Society' in 1889 and in a voluminous memoir which appeared in the 'Philosophical Transactions' in 1893. It was unfortunate, for various reasons, that the publication of this Memoir had been so long delayed.

The anatomical relations which form the subject of this joint paper are of no little interest. They were first described in 1820 by Weber, who showed that in the Siluridæ and Cyprinidæ a short chain of bones intervenes, on either side, between the anterior part of the air-bladder and the auditory organ, and regarded the air-bladder as thus accessory to the function of hearing. Bridge and Haddon, depending to a considerable extent on a part which had been purchased of Dr. Bleeker's well-known collection of East Indian Fishes, added greatly to our knowledge of the "Weberian ossicles" in the Siluridæ. No less than 100 species, referred to 51 genera, were examined. The view that the fishes possessing these ossicles are related to one another was fully confirmed, since the agreement throughout the Ostariophysi in regard to the ossicles is too detailed to permit of explanation on any other theory. The Weberian mechanism includes modifications of the auditory organ, of the air-bladder, and of the anterior part of the vertebral column. The axial skeleton in this region has probably given rise to the ossicles, the homologies of which are carefully considered. A well-reasoned discussion of their functions follows.

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* 'Science' (N.S.), vol. 21, 1905, p. 661.

It is a striking fact, as is pointed out by the authors, that "the presence of a Weberian mechanism is characteristic of nearly all the dominant families of fresh-water Teleostei"; and it might be supposed that its possessors derive "some exceptional advantage therefrom." What that advantage may be is not perfectly certain, in the absence of sufficient experimental evidence. The authors give weighty reasons for believing that the mechanism is not for the appreciation of the small vibrations which are concerned in producing an auditory stimulus, and conclude that it is probably to acquaint the fish, through the auditory organ, with the varying degrees of tension of the gaseous contents of the air-bladder due to variations in the height of the superincumbent column of water. It may be remarked that this manometer-like function of the Weberian mechanism has recently been supported by Thilo, who gives figures* showing the different positions assumed by the ossicles in a Carp with the air-bladder respectively tense and flaccid.

Of Bridge's other special Memoirs it is perhaps unnecessary to speak in detail, since they can hardly appeal to any except Vertebrate Morphologists. They include papers on the Skulls of *Amia*,† *Polypterus*, *Lepidosiren*, and *Osteoglossum*, on the Osteology of *Polyodon*, on the Mesial Fins of Ganoids and Teleosts, and on the Air-bladder and Auditory Organ of *Notopterus*.

Bridge's latest work was his article on Fishes in vol. 7 of 'The Cambridge Natural History,' and to this he devoted his best efforts. The writing of that article gave him the opportunity of putting together and making available for others his wide knowledge of the Morphology of Fishes, though other parts of the subject were by no means neglected. Although some of his statements have been criticised, the chapters contributed by Bridge to this volume are a most valuable summary of a very difficult subject. As one of the editors of the volume in question, the writer of this notice had many opportunities of admiring Bridge's devotion to his work, the trouble he would take to avoid carelessness or inaccuracy, and his keen desire to make his article as good as possible, without thought of any other considerations. He was, perhaps, unduly critical of his own performances, and was not satisfied to publish until he had made himself certain that he had done everything in his power to arrive at a correct result. This attitude of mind and the continued ill-health from which he suffered were no doubt responsible for the fact that his list of published papers is not a long one; though, on the other hand, there is probably nothing which he wrote that will not repay perusal. He was of a reserved nature, and there were not many persons who were admitted to his confidence. But those who knew him had a high respect for his thoroughness and his disinterested singleness of purpose. He died, unmarried, on June 29, 1909.

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* 'Zool. Anzeiger,' vol. 32, 1908, p. 781.

† A list of the more important contributions is given at the end of this notice.

List of the Principal Publications of T. W. Bridge.

1. "The Cranial Osteology of *Amia calva*," 'Journ. Anat. and Physiol.,' vol. 11, 1877, p. 605.

2. "On the Osteology of *Polyodon folium*," 'Phil. Trans.,' vol. 169 (for 1878), 1879, p. 683.

3. "Pori abdominales of Vertebrata," 'Journ. Anat. and Physiol.,' vol. 14, 1879, p. 81.

4. "Some Points in the Cranial Anatomy of *Polypterus*," 'Proc. Birmingham Phil. Soc.,' vol. 6, Sessions 1887—88, 1888—89, p. 118.

5. "The Air-bladder in certain Siluroid Fishes," '*t. cit.*, p. 131.

6. (With A. C. Haddon.) "The Air-bladder and Weberian Ossicles in the Siluroid Fishes," 'Roy. Soc. Proc.,' vol. 46, 1890, p. 309; vol. 52, 1893, p. 139; 'Phil. Trans.,' vol. 184, B, 1894, p. 65.

7. "On certain Features in the Skull of *Osteoglossum formosum*," 'Proc. Zool. Soc.,' 1895, p. 302.

8. "The Mesial Fins of Ganoids and Teleosts," 'J. Linn. Soc. (Zool.),' vol. 25, 1896, p. 530.

9. "On the Presence of Ribs in *Polyodon* (*Spatularia*) *folium*," 'Proc. Zool. Soc.,' 1897, p. 722.

10. "On the Morphology of the Skull in the Paraguayan *Lepidosiren* and in other Dipnoids," 'Trans. Zool. Soc.,' vol. 14, 1898, p. 325.

11. "The Air-bladder and its Connection with the Auditory Organ in *Notopterus borneensis*," 'J. Linn. Soc. (Zool.),' vol. 27, 1899—1900, p. 503.

12. Art.: "Fishes" (exclusive of the Systematic Account of Teleostei), 'The Cambridge Natural History,' vol. 7, 1904.

S. F. H.

INDEX TO VOL. LXXXII. (B)

Address of President, November 30, 1909 (Geikie), 151.
Adrenal medulla and chromaphil tissues (Vincent), 502.
Agglutinin, absorption by bacteria, and application of physico-chemical laws thereto (Dreyer and Douglas), 185 ; —— adsorption by animal charcoal, velocity of reaction in (Dreyer and Douglas), 168.
Alcoholic fermentation, function of phosphates in (Harden and Young), 321.
Amakebe, a disease of calves in Uganda (Bruce and others), 256.
Arbuckle (H. E.) Some Experiments with the Venom of *Causus rhombeatus*, 144.
Armstrong (H. E. and E. F.) The Origin of Osmotic Effects. III.—The Function of Hormones in stimulating Enzymic Change in Relation to Narcosis and the Phenomena of Degenerative and Regenerative Change in Living Structures, 588.
Armstrong (H. E.) and Horton (E.) Studies on Enzyme Action. XIII.—Enzymes of the Emulsin Type, 349.
Autotoxæmia and infection (Hort), 529.

Bashford (E. F.) and Russell (B. R. G.) Further Evidence on the Homogeneity of the Resistance to the Implantation of Malignant New Growths, 298.
Bateman (H. R.) See Bruce (Sir D.) and others.
Blood from diseases in man, hæm-agglutinins, hæm-opsonins, etc., in (Dudgeon and Wilson), 67.
Blood, inorganic composition of, and its origin (Macallum), 602.
Blood volume of mammals, and relation to body-weight and surface-area expressed in a formula (Dreyer and Ray), 545.
Bolton (C.) Further Observations on the Pathology of Gastric Ulcer (Progress Report), 233.
Bottomley (W. B.) The Assimilation of Nitrogen by certain Nitrogen-fixing Bacteria in the Soil, 627.
Brain, occurrence of mesocœlic recess in (Dendy and Nicholls), 515.
Bridge (T. W.) Obituary notice of, vii.
Bruce (Sir D.) and others. Sleeping Sickness in Uganda.—Duration of Infectivity of *Glossina palpalis* after the Removal of the Lake-shore Population, 56 ; —— *Glossina palpalis* as a Carrier of *Trypanosoma vivax* in Uganda, 63 ; —— Amakebe : a Disease of Calves in Uganda, 256 ; —— The Development of Trypanosomes in Tsetse Flies, 368 ; —— Trypanosome Diseases of Domestic Animals in Uganda. I.—*Trypanosoma pecorum*, 468 ; —— "Muhinyo," a Disease of Natives in Uganda, 485 ; —— Experiments to ascertain if Cattle may act as a Reservoir of the Virus of Sleeping Sickness (*Tryp. gambiense*), 480 ; —— The Natural Food of *Glossina palpalis*, 490 ; —— Mechanical Transmission of Sleeping Sickness by the Tsetse Fly, 498.

Caffeine and theobromine, comparative toxicity of (Veley and Waller), 568.
Cancer, reactions to implantation of, after inoculation of living and disintegrated cells (Haaland), 293.
Carbon dioxide assimilation in leaves, increase of weight as measure of (Thoday), 1.
Causus rhombeatus, experiments with venom of (Arbuckle), 144.
Cerebral cortex of Echidna, cell lamination of (Schuster), 113.

Chapman (H. G.) On the Weight of Precipitate obtainable in Precipitin Interactions, 398.
Cholesterol and cholesterol esters, quantity in blood of rabbits on varying diets (Fraser and Gardner), 559.
Chromaphil tissues and adrenal medulla (Vincent), 502.
Cole (F. J.) Tone Perception in *Gammarus pulex*, 391.
Colour perception, relation of light perception to (Edridge-Green), 458.
Cramer (W.) and Pringle (H.) Contributions to the Biochemistry of Growth.—The Total Nitrogen Metabolism of Rats bearing Malignant New Growths, 307.
Cranial nerves, receptors and afferents of the third, fourth, and sixth (Tozer and Sherrington), 450.
Croonian lecture (Klebs), 547.
Cushny (A. R.) See Thomson and Cushny.

Dallinger (W. H.) Obituary notice of, iv.
Dendy (A.) On the Structure, Development, and Morphological Interpretation of the Pineal Organs and Adjacent Parts of the Brain in the Tuatara (*Sphenodon punctatus*), 629.
Dendy (A.) and Nicholls (G. E.) On the Occurrence of a Mesocœlic Recess in the Human Brain, and its Relation to the Sub-Commissural Organ of Lower Vertebrates, 515.
Doncaster (L.) Gametogenesis of the Gall-fly, *Neuroterus lenticularis*.—Part I, 88.
Douglas (C. G.) and Haldane (J. S.) The Causes of Absorption of Oxygen by the Lungs (Preliminary Communication), 331.
Douglas (J. S. C.) See Dreyer and Douglas.
Dreyer (G.) and Douglas (J. S. C.) The Velocity of Reaction in the "Absorption" of Specific Agglutinins by Bacteria, and in the "Adsorption" of Agglutinins, Trypsin, and Sulphuric Acid by Animal Charcoal, 168; —— On the Absorption of Agglutinin by Bacteria, and the Application of Physico-chemical Laws thereto, 185.
Dreyer (G.) and Ray (W.) The Blood Volume of Mammals as Determined by Experiments upon Rabbits, Guinea-Pigs, and Mice, and its Relationship to the Body Weight and Surface Area expressed in a Formula, 545.
Drugs, rate of action of, upon muscle as function of temperature (Veley and Waller), 205.
Dudgeon (L. S.), Panton (P. N.), and Wilson (H. A. F.) The Influence of Bacterial Endotoxins on Phagocytosis (Preliminary Report), 406.
Dudgeon (L. S.) and Wilson (H. A. F.) On the Presence of Hæm-agglutinins, Hæm-opsonins, and Hæmolysins in the Blood obtained from Infectious and Non-infectious Diseases in Man (Third Report), 67.

Echidna, cell lamination of cortex of, and enumeration of fibres in cranial nerves (Schuster), 113.
Edridge-Green (F. W.) The Relation of Light Perception to Colour Perception, 458.
Electrification, opposite, produced by animal and vegetable life (Thornton), 638.
Enzyme action, studies on. XIII.—Enzymes of the emulsin type (Armstrong and Horton), 349.
Enzymic change, function of hormones in stimulating (Armstrong), 588.

Fishes, caudal fin of (Whitehouse), 134.
FitzGerald (M. P.) Preliminary Note on the Origin of the Hydrochloric Acid in the Gastric Tubules, 346.
Formaldehyde in green plants, photochemical formation of (Schryver), 226.

Fraser (M. T.) and Gardner (J. A.) The Origin and Destiny of Cholesterol in the Animal Organism. Part VII.—On the Quantity of Cholesterol and Cholesterol Esters in the Blood of Rabbits fed on Diets containing Varying Amounts of Cholesterol, 559.

Galactose, fermentation of, by yeast and yeast-juice (Harden and Norris), 645.
Gall-fly (*Neuroterus lenticularis*), gametogenesis of (Doncaster), 88.
Gammarus pulex, tone perception in (Cole), 391.
Gardner (J. A.) See Fraser and Gardner.
Gastric tubules, origin of the hydrochloric acid in (FitzGerald), 346 ; —— ulcer, further observations on pathology of (Bolton), 233.
Geikie, Sir A. Presidential Address, November 30, 1909, 151.
Glossina palpalis as carrier of *Trypanosoma vivax* (Bruce and others), 63 ; —— natural food of (Bruce and others), 490.
Growth, contributions to biochemistry of (Cramer and Pringle), 307.

Haaland (M.) The Contrast in the Reactions to the Implantation of Cancer after the Inoculation of Living and Mechanically Disintegrated Cells, 293.
Hæm-agglutinins, hæm-opsonins, and hæmolysins in blood from diseases in man (Dudgeon and Wilson), 67.
Hæmolytic methods, recognition of individual by (Todd and White), 416.
Haldane (J. S.) See Douglas and Haldane.
Halliburton (W. D.) See Mott, Schuster, and Halliburton.
Hamerton (A. E.) See Bruce (Sir D.) and others.
Harden (A.) and Norris (R. V.) The Fermentation of Galactose by Yeast and Yeast-juice (Preliminary Communication), 645.
Harden (A.) and Young (W. J.) The Alcoholic Ferment of Yeast-juice. Part V.—The Function of Phosphates in Alcoholic Fermentation, 321.
Hormones, function of, in stimulating enzymic change (Armstrong), 588.
Hort (E. C.) Autotoxæmia and Infection, 529.
Horton (E.) See Armstrong (H. E.) and Horton.
Hydrochloric acid in gastric tubules, origin of (FitzGerald), 346.
Hydrocyanic acid in tissues, new method for quantitative estimation (Waller), 574.

Klebs (G.) Alterations in the Development and Forms of Plants as a Result of Environment (Croonian Lecture), 547.

Leaves, carbon dioxide assimilation in (Thoday), 1.
Light perception, relation of, to colour perception (Edridge-Green), 458.
Lignite of Bovey Tracey (Reid), 650.
Lungs, causes of absorption of oxygen by (Douglas and Haldane), 331.

Macallum (A. B.) The Inorganic Composition of the Blood in Vertebrates and Invertebrates, and its Origin, 602.
Mackie (F. P.) See Bruce (Sir D.) and others.
Magnetic field, physiological effect of an alternating (Thompson), 396.
Malignant new growths, relative sizes of organs of rats, etc., bearing (Medigreceanu), 286 ; —— homogeneity of resistance to implantation of (Bashford and Russell), 298 ; —— nitrogen metabolism of rats bearing (Cramer and Pringle), 307.
Marmoset, cortical lamination and localisation in brain of (Mott and others), 124.
Medals, award of, for 1909, 165.
Medigreceanu (F.) On the Relative Size of the Organs of Rats and Mice bearing Malignant New Growths, 286.

Metabolism, total nitrogen, of rats bearing malignant new growths (Cramer and Pringle), 307.

Metals, germicidal action of, and relation to production of peroxide of hydrogen (Rankin), 78.

Minchin (E. A.) and Thomson (J. D.) The Transmission of *Trypanosoma lewisi* by the Rat-flea (*Ceratophyllus fasciatus*). Preliminary communication, 273.

Mott (F. W.), Schuster (E.), and Halliburton (W. D.) Cortical Lamination and Localisation in the Brain of the Marmoset, 124.

"Muhinyo," a disease of natives in Uganda (Bruce and others), 485.

Muscle, action of nicotine, etc., on (Veley and Waller), 333 ; —— rate of action of drugs on, as function of temperature (Veley and Waller), 205.

Nicholls (G. E.) See Dendy and Nicholls.

Nicotine and other pyridine bases, action of, upon muscle (Veley and Waller), 333.

Nitrogen assimilation by certain bacteria in soil (Bottomley), 627.

Norris (R. V.) See Harden and Norris.

Obituary notices :—
    Bridge (T. W.), vii.
    Dallinger (W. H.), iv.
    Pflüger (E. F. W.), i.

Osmotic effects, origin of, III (Armstrong), 588.

Panton (P. N.) See Dudgeon, Panton, and Wilson.

Pflüger (E. F. W.) Obituary notice of, i.

Phagocytosis, influence of bacterial endotoxins on (Dudgeon, Panton, and Wilson), 406.

Plants, alteration in development and forms of, as a result of environment (Klebs), 547.

Precipitin interactions, weight of precipitate obtainable in (Chapman), 398.

Pringle (H.) See Cramer and Pringle.

Rankin (A. C.) The Germicidal Action of Metals and its Relation to the Production of Peroxide of Hydrogen, 78.

Ray (W.) See Dreyer and Ray.

Reid (C. and E. M.) The Lignite of Bovey Tracey, 650.

Reissner's fibre, distribution in vertebrate series, and function (Dendy and Nicholls), 515.

Ross (R.) and Thomson (D.) A Case of Sleeping Sickness studied by Precise Enumerative Methods : Regular Periodical Increase of the Parasites disclosed, 411.

Russell (B. R. G.) See Bashford and Russell.

Schryver (S. B.) The Photochemical Formation of Formaldehyde in Green Plants, 226.

Schuster (E.) Preliminary Note upon the Cell Lamination of the Cerebral Cortex of Echidna, with an Enumeration of the Fibres in the Cranial Nerves, 113 ; —— see also Mott, Schuster, and Halliburton.

Sherrington (C. S.) See Tozer and Sherrington.

Sleeping sickness in Uganda—duration of infectivity of Glossina (Bruce and others), 56 ; —— case of, studied by precise enumerative methods (Ross and Thomson), 411 ; —— cattle as reservoir of virus of (Bruce and others), 480 ; —— mechanical transmission of, by the tsetse fly (Bruce and others), 498.

Soil, assimilation of nitrogen by bacteria in (Bottomley), 627.

Sphenodon, pineal organs and adjacent parts of brain in (Dendy), 629.

Stovaine and cocaine, comparative action of, on contractility of isolated muscle (Veley and Waller), 147.

Sykes (M. G.) The Anatomy and Morphology of the Leaves and Inflorescences of *Welwitschia mirabilis*, 625.

Tetrachlorethane and trichlorethylene, physical and physiological properties of (Veley), 217.
Theobromine and caffeine, comparative toxicity of (Veley and Waller), 568.
Thoday (D.) Experimental Researches on Vegetable Assimilation and Respiration. V.—A Critical Examination of Sachs' Method for using Increase of Dry Weight as a Measure of Carbon Dioxide Assimilation in Leaves, 1 ; —— Experimental Researches on Vegetable Assimilation and Respiration. VI.—Some Experiments on Assimilation in the Open Air, 421.
Thompson (F. D.) The Thyroid and Parathyroid Glands throughout Vertebrates, 389.
Thompson (S. P.) A Physiological Effect of an Alternating Magnetic Field, 396.
Thomson (D.) See Ross and Thomson.
Thomson (J. D.) See Minchin and Thomson.
Thomson (J. D.) and Cushny (A. R.) On the Action of Antimony Compounds in Trypanosomiasis in Rats : being a Report to a Sub-Committee of the Tropical Diseases Committee of the Royal Society, 249.
Thornton (W. M.) The Opposite Electrification produced by Animal and Vegetable Life, 638.
Thyroid and parathyroid glands throughout vertebrates (Thompson), 389.
Todd (C.) and White (R. G.) On the Recognition of the Individual by Hæmolytic Methods (Preliminary Communication), 416.
Tone perception in *Gammarus pulex* (Cole), 391.
Tozer (F. M.) and Sherrington (C. S.) Receptors and Afferents of the Third, Fourth, and Sixth Cranial Nerves, 450.
Trypanosoma lewisi, transmission of, by the rat-flea (Minchin and Thomson), 273.
Trypanosoma vivax, *Glossina palpalis* as carrier of (Bruce and others), 63.
Trypanosome diseases of domestic animals in Uganda. I.—*Trypanosoma pecorum* (Bruce and others), 468.
Trypanosomes, development of, in tsetse flies (Bruce and others), 368.
Trypanosomiasis in rats, action of antimony compounds in (Thomson and Cushny), 249.

Vegetable assimilation and respiration, experimental researches on (Thoday), 1, 421.
Veley (V. H.) An Examination of the Physical and Physiological Properties of Tetrachlorethane and Trichlorethylene, 217.
Veley (V. H.) and Waller (A. D.) On the Comparative Action of Stovaine and Cocaine as measured by their Direct Effect upon the Contractility of Isolated Muscle, 147 ; —— Observations on the Rate of Action of Drugs (Alcohol, Chloroform, Quinine, Aconitine) upon Muscle as a Function of Temperature, 205 ; —— The Action of Nicotine and other Pyridine Bases upon Muscle, 333 ; —— On the Comparative Toxicity of Theobromine and Caffeine, as measured by their Direct Effect upon the Contractility of Isolated Muscle, 568.
Vincent (Swale). The Chromaphil Tissues and the Adrenal Medulla, 502.

Waller (A. D.) The Action of Nicotine and other Pyridine Bases upon Muscle, 333 ; —— A new Method for the Quantitative Estimation of Hydrocyanic Acid in Vegetable and Animal Tissues, 574 ; —— See also Veley and Waller.
Welwitschia mirabilis, anatomy and morphology of (Sykes), 625.
White (R. G.) See Todd and White.

Whitehouse (R. H.) The Caudal Fin of Fishes (Preliminary Paper), 134.
Wilson (H. A. F.) See Dudgeon and Wilson ; and Dudgeon, Panton, and Wilson.

Yeast and yeast-juice, fermentation of galactose by (Harden and Norris), 645.
Yeast-juice, alcoholic ferment of. Part V (Harden and Young), 321.
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ON THE SCATTERING OF HOMOGENEOUS β-RAYS AND THE NUMBER OF ELECTRONS IN THE ATOM. By J. A. CROWTHER, M.A., Fellow of St. John's College, Cambridge 226

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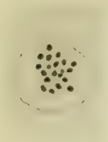

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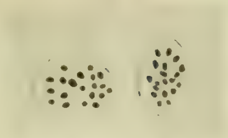

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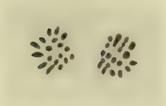

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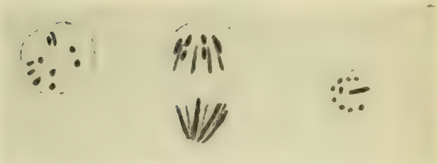

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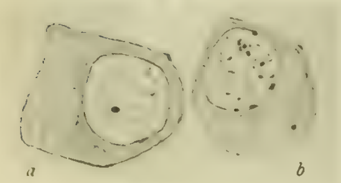

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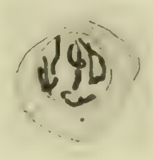

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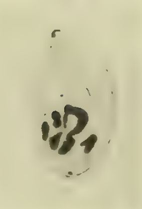

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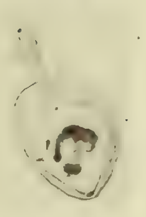

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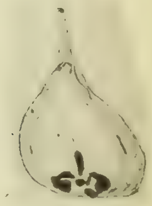

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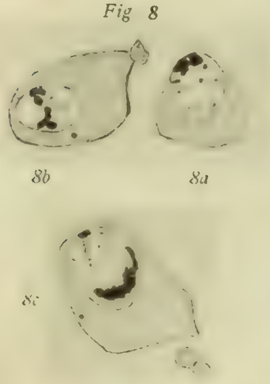

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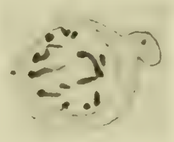

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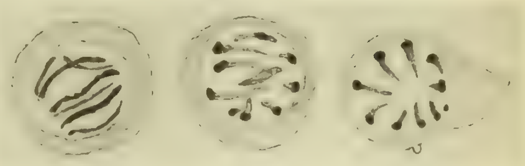

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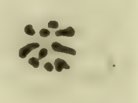


Fig. 11

Fig. 12

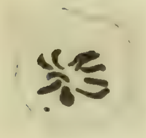
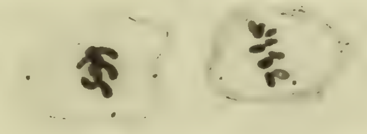


Fig. 13



13a 13b

Fig. 14a

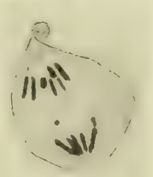


Fig. 14b

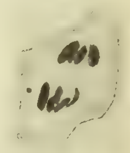
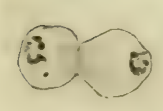


Fig. 15a

Fig. 15b



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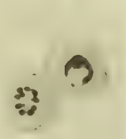

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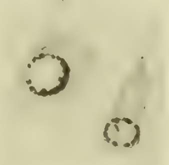

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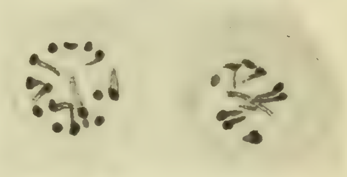
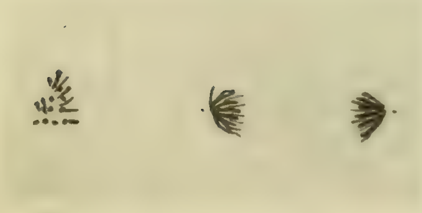


18a 18b

Fig. 19



19a 19b

Fig. 20

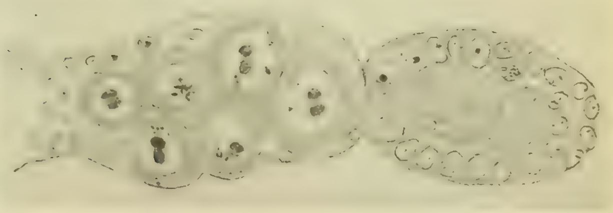


Fig. 21

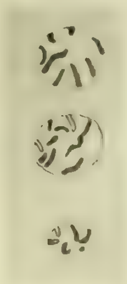


Fig 22

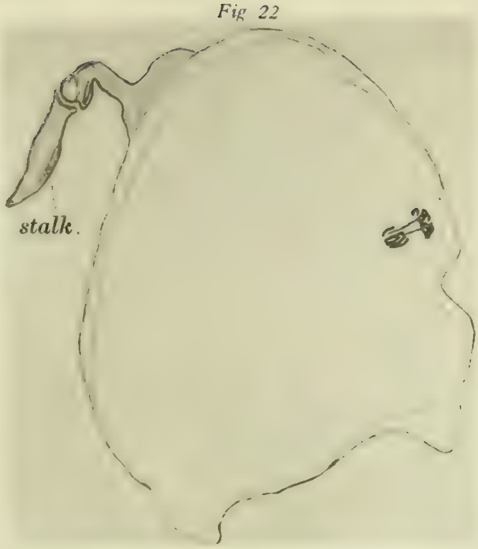
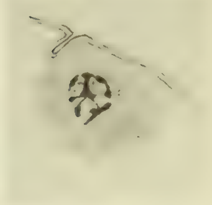


Fig. 23



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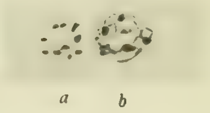

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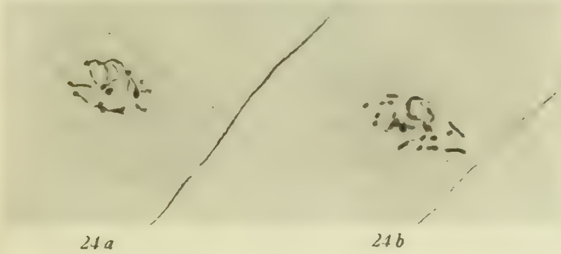
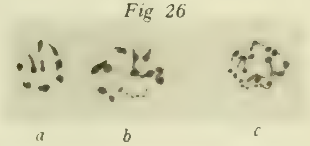


Fig 26



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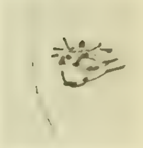

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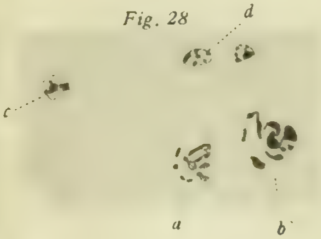
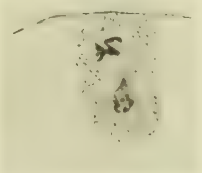


Fig 29



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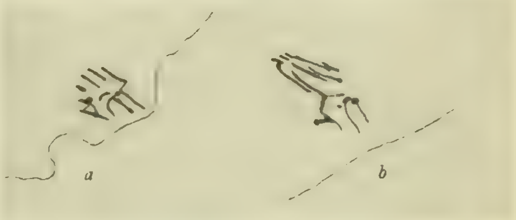
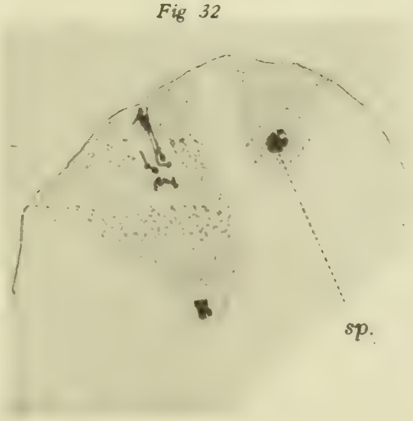


Fig 32



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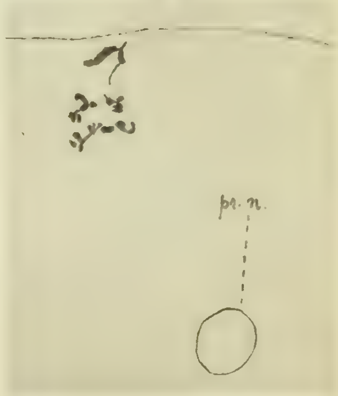

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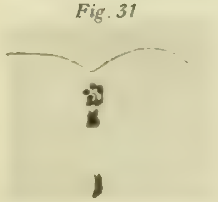

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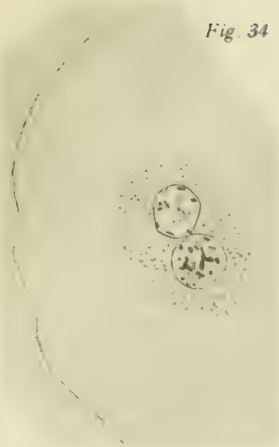


Fig 35

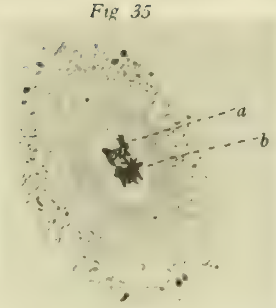


Fig. 36a

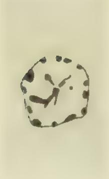
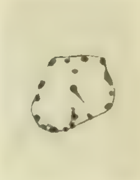


Fig. 36b



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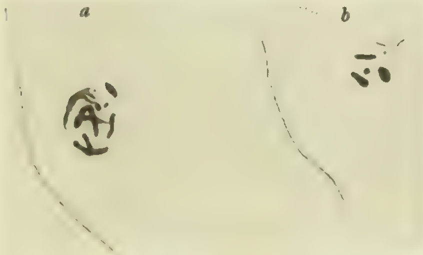


Fig. 40

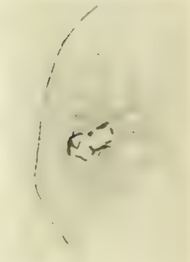
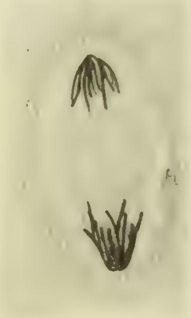


Fig 37



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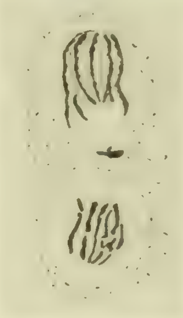

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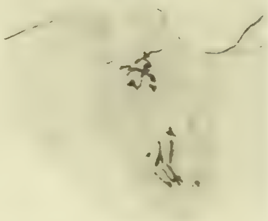
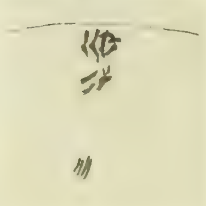


Fig. 42



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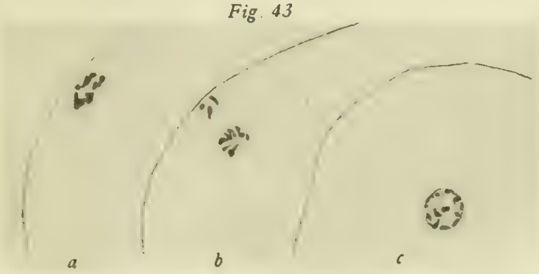
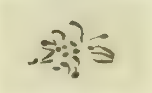


Fig 44a



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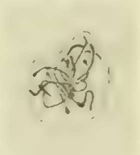


Fig. 44b

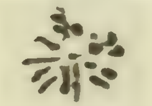


Fig 46

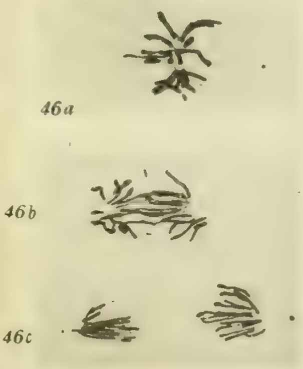


Fig. 47

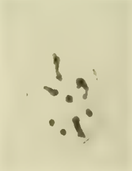


Fig. 48a

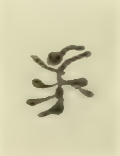


Fig. 48b

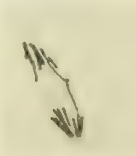
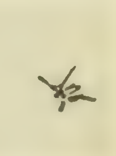


Fig. 48c



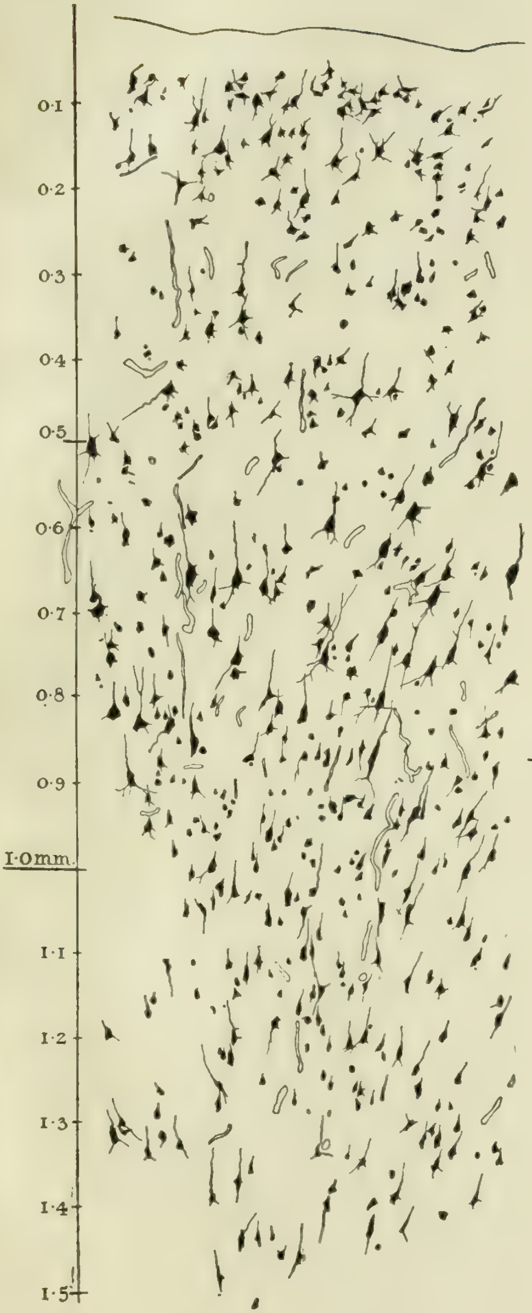

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Fig. 3.—(Type 2) × 190.

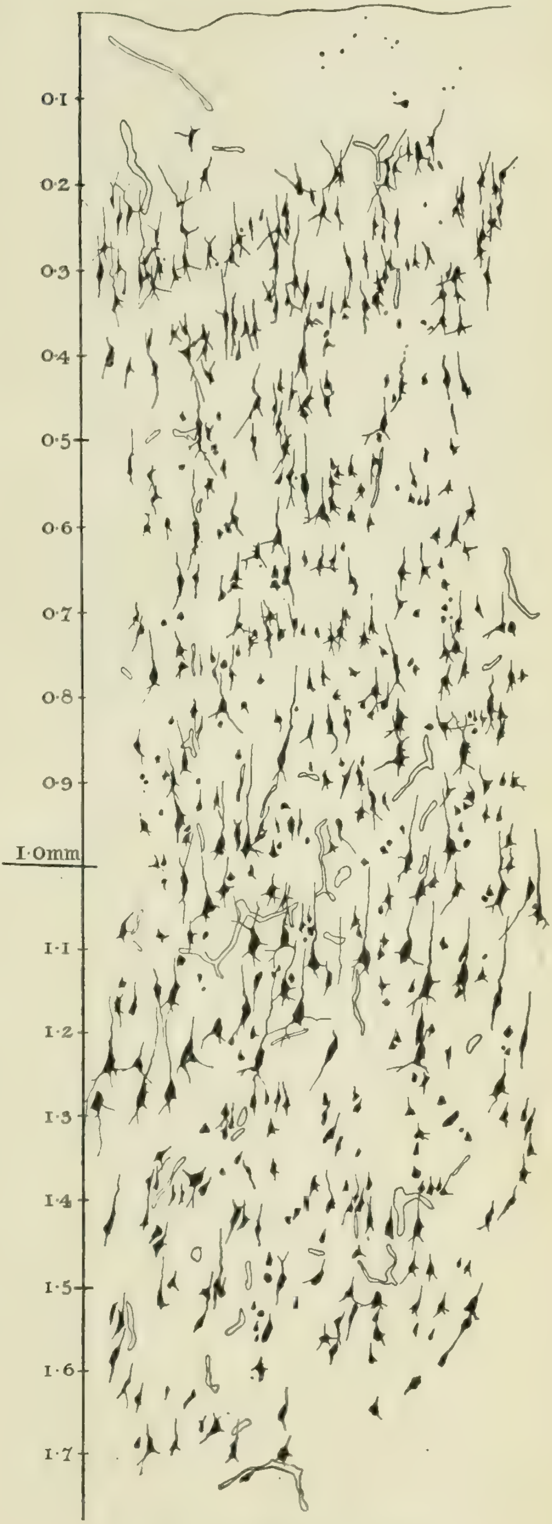
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Fig. 4.—(Type 3) × 190.

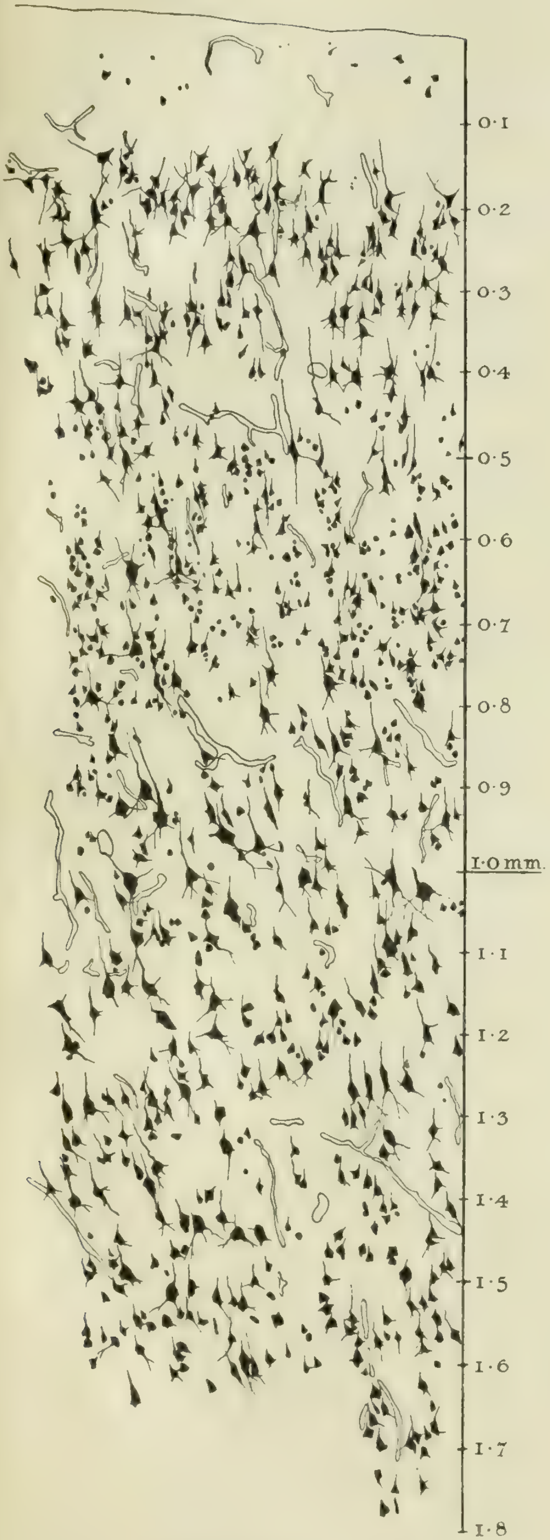
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Fig. 5.—(Type 4) × 190.

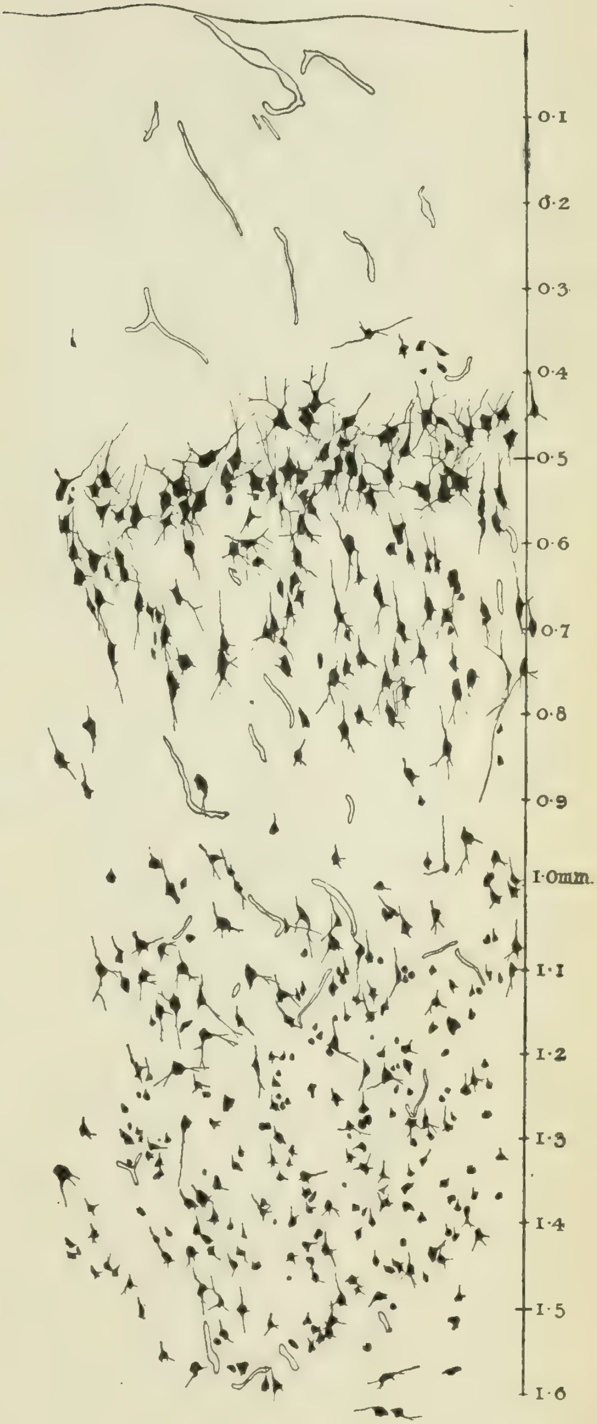
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Fig. 6.—(Type 5) × 190.

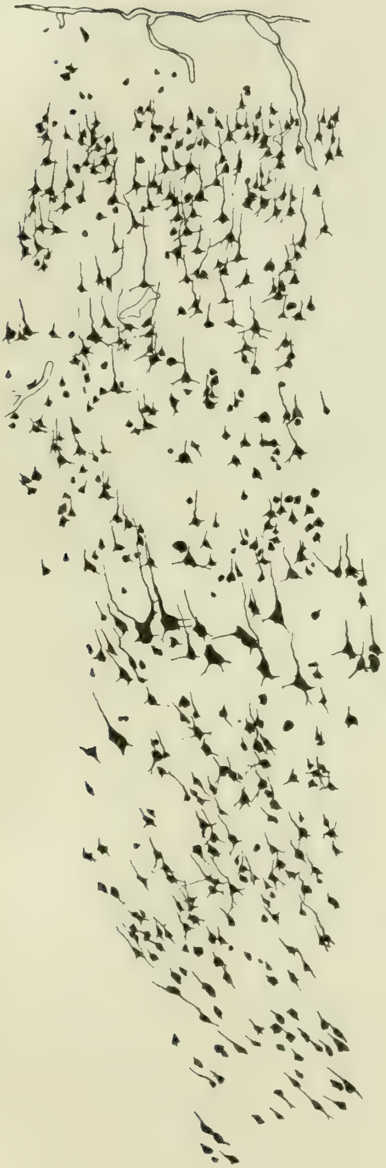


FIG. 3.—Motor cortex A.

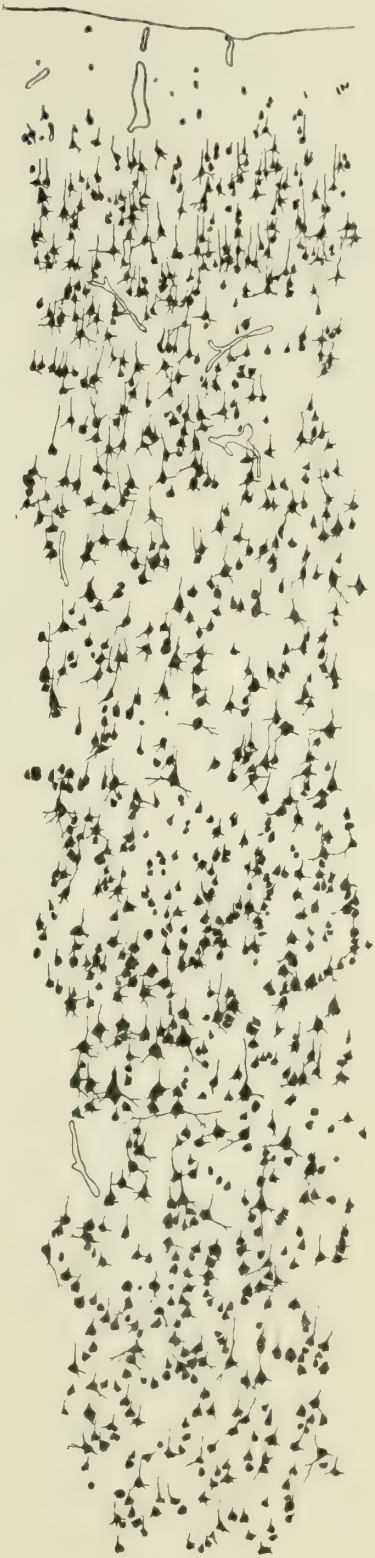


FIG. 4.—Motor cortex B.

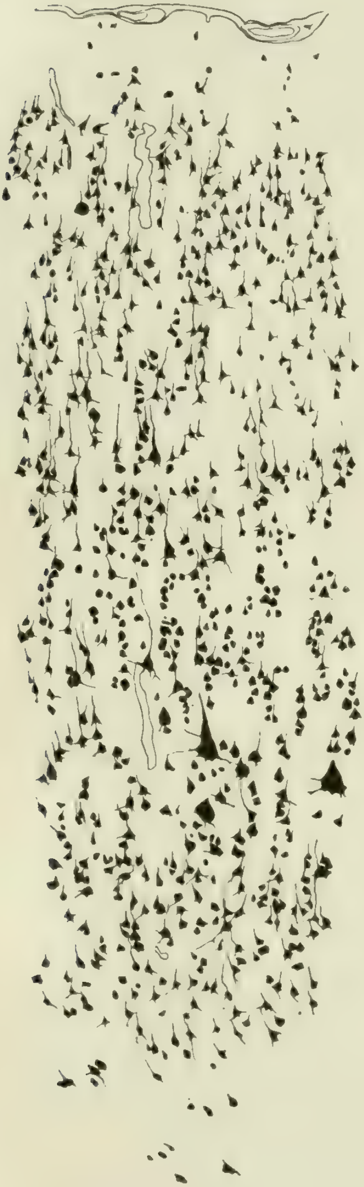


FIG. 5.—Post-central cortex.

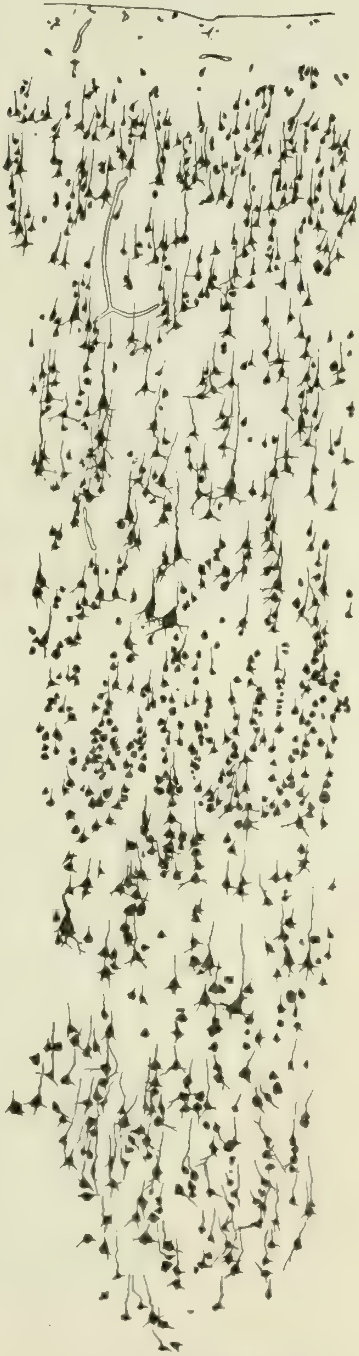


FIG. 6.—Temporal cortex.

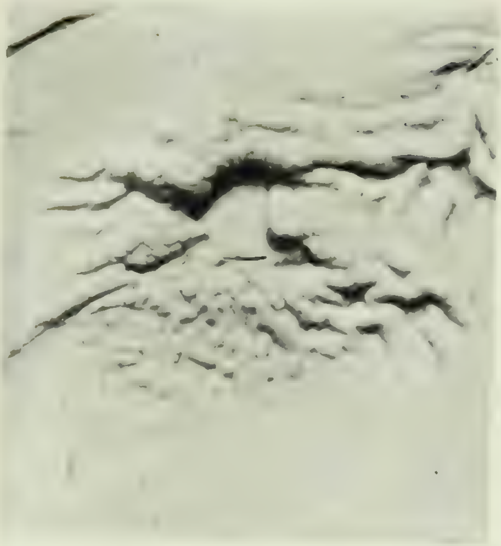


FIG. 1, A.

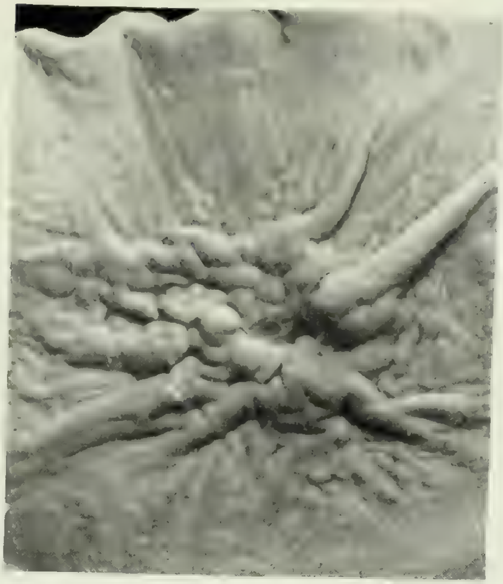


FIG. 1, B.

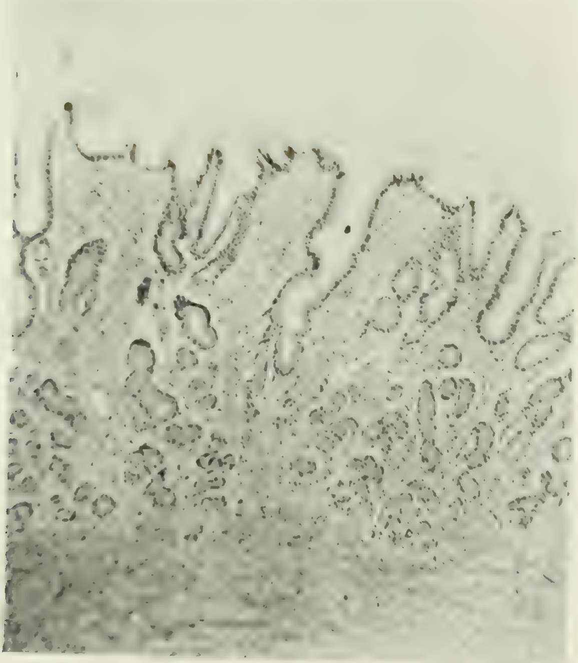


FIG. 2.

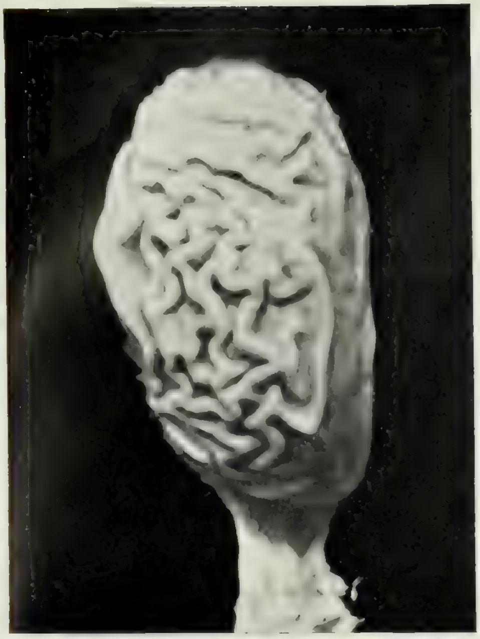


FIG. 3, A.

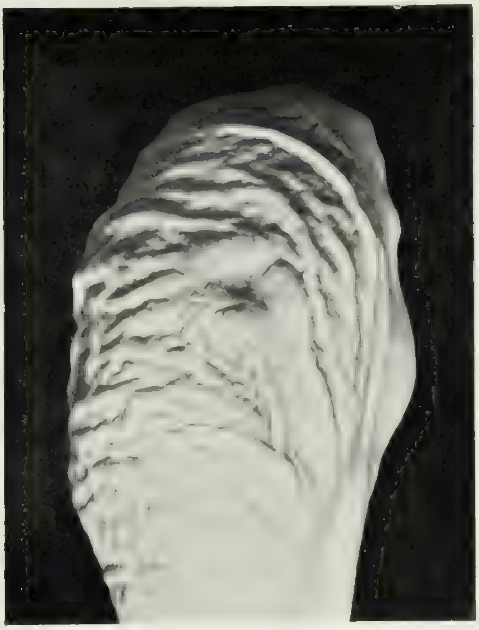


FIG. 3, B.

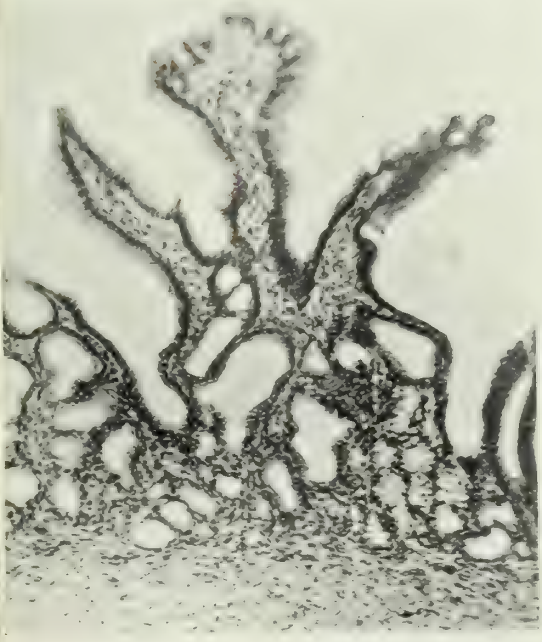


FIG. 4.

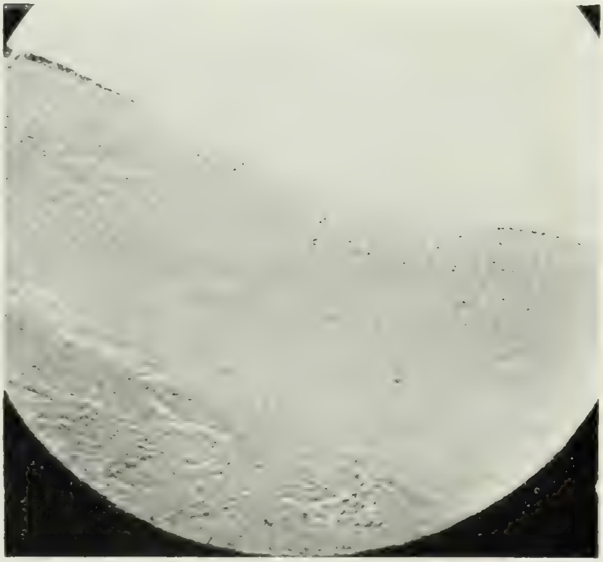


FIG. 5.

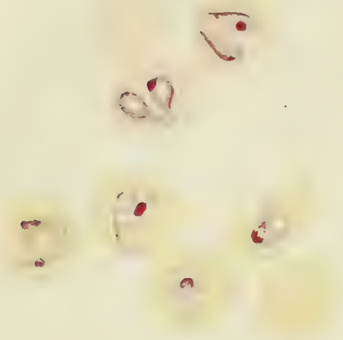


Fig. 1.

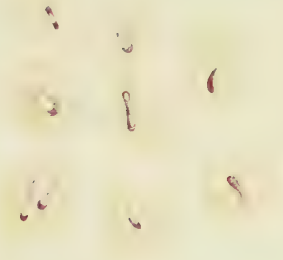


Fig. 2.

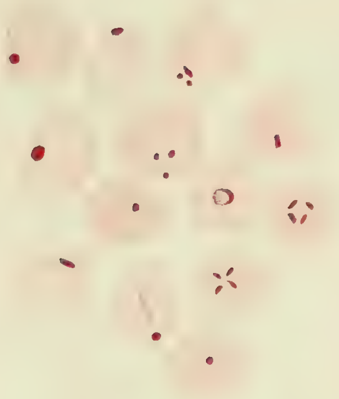


Fig. 3.

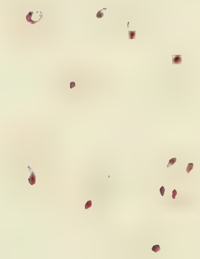


Fig. 4.

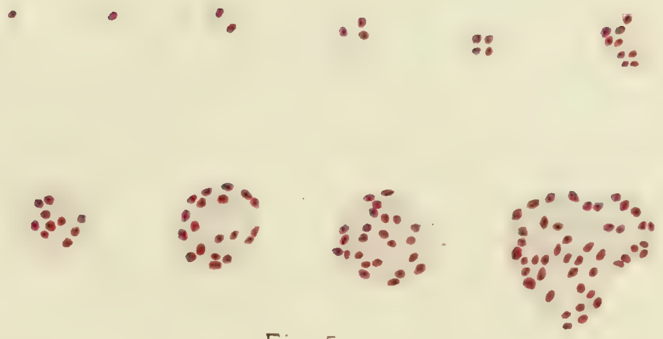
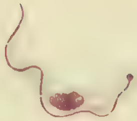
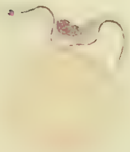


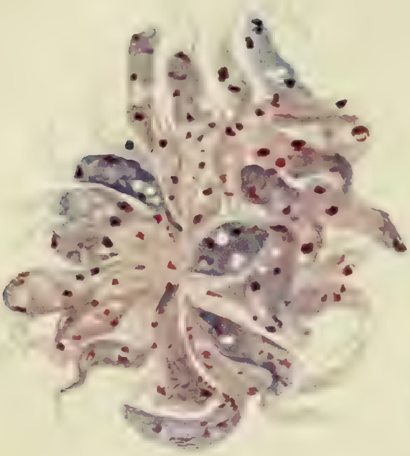
Fig 5.

Bruce & others

Roy. Soc. Proc. B. vol. 82, Pl. 1







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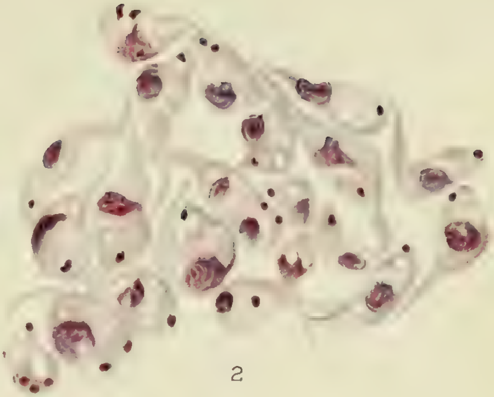

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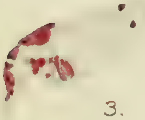

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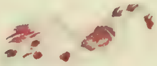

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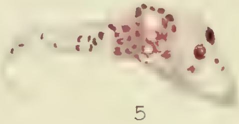

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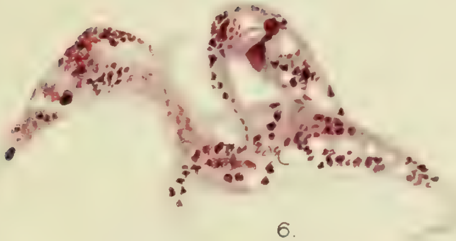

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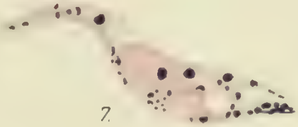

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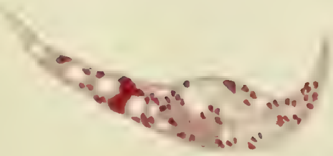

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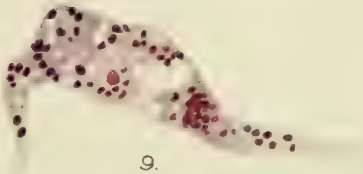

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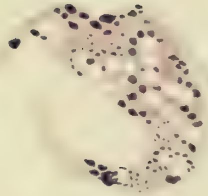

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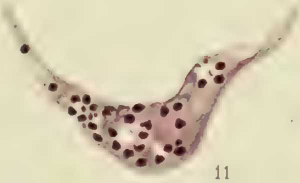

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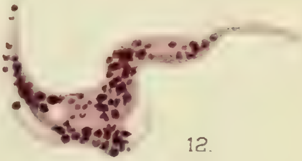

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Fig. 1

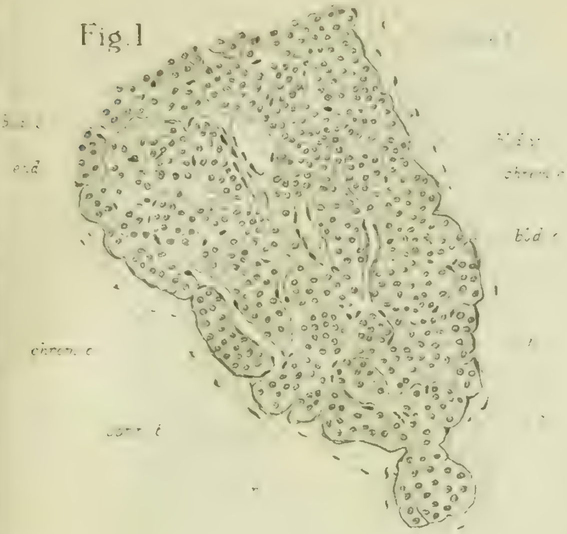
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Fig. 2

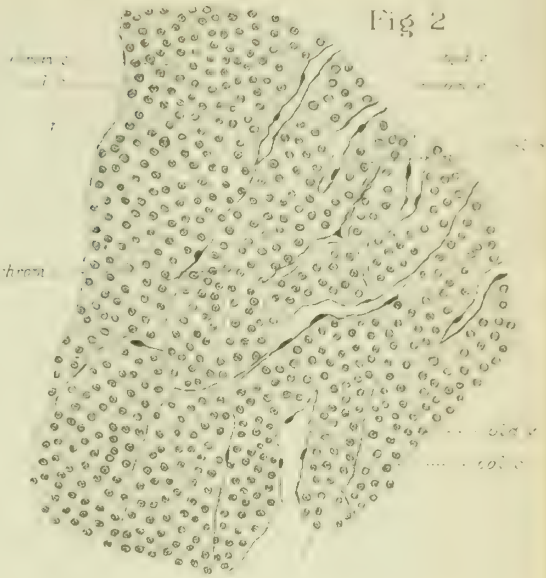
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Fig. 3

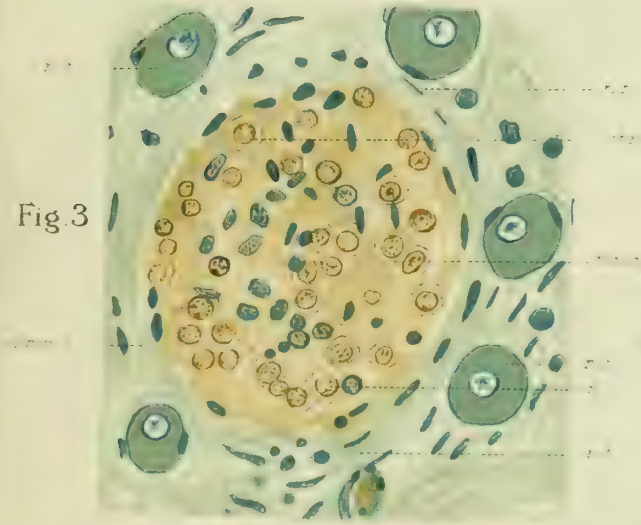
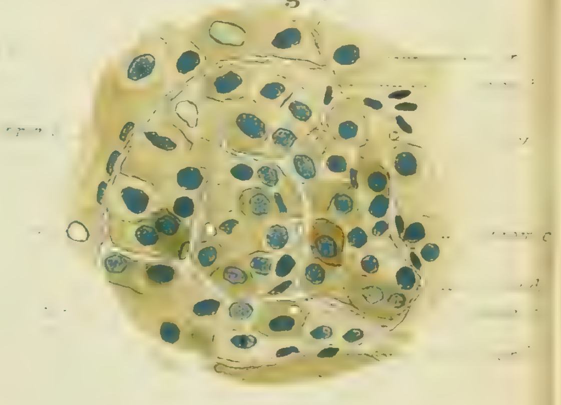


Fig. 4



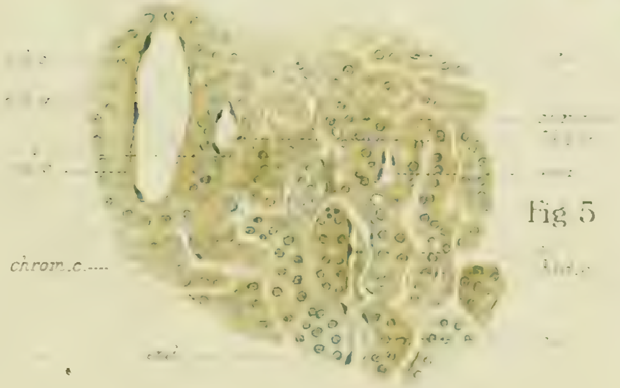

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Fig. 5

Purbrook & Eyres, Lith.

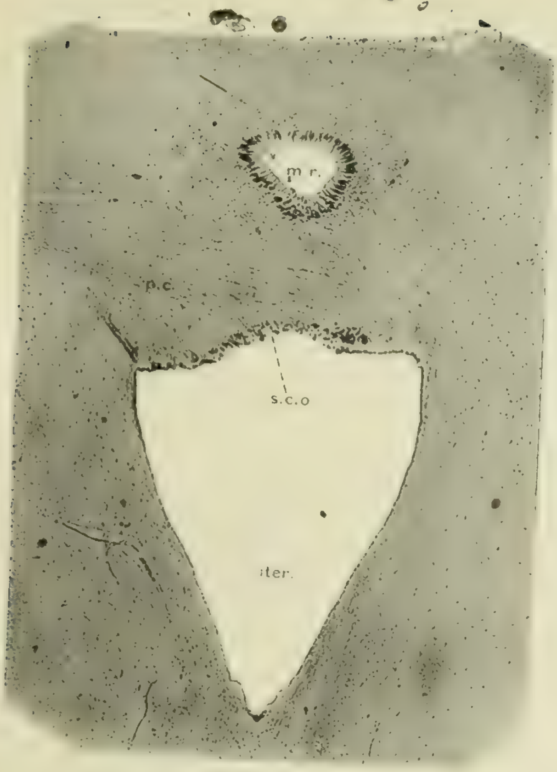
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Fig. 1.

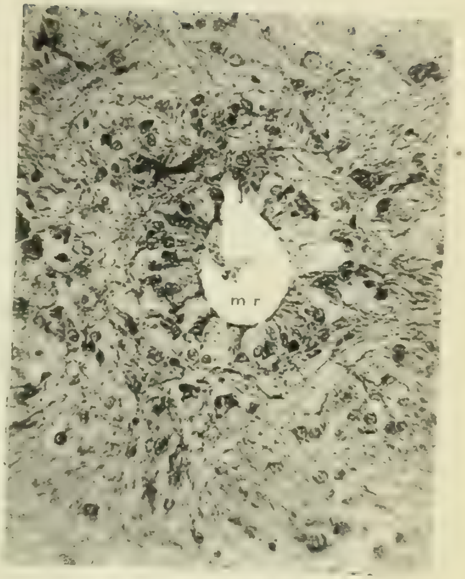

Fig. 3.

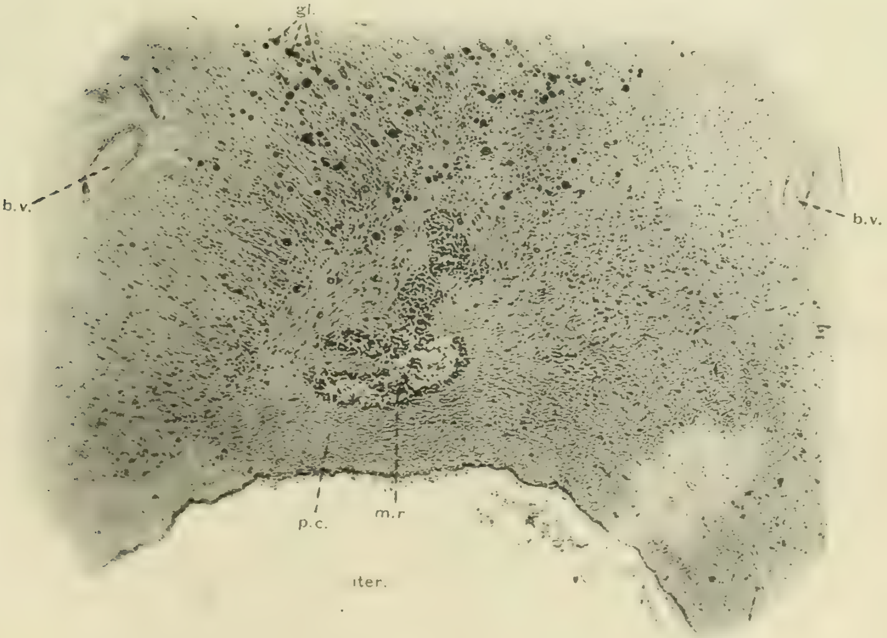
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Fig. 2

W. H. Row, *Photo*

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